The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Chemistry and Biology of Lipids

XII. Group B Lipoid of Human Pancreas

First Paper

By

Masahiro Tokura
(登倉 正博)

(From the Medico-chemical Institute, Tohoku University, Sendai; Director: Prof. H. Masamune)

(Received for publication, April 8, 1952)

The procedure employed in preparing the Group **A** lipoid of human pancreas[1] was applied to the same organ of persons belonging to Group **B**. Here again, an electrophoretically homogeneous product was obtained, which proved identical with the Group **A** lipoid in so far as N, P and carbohydrate content and solubilities concern.

Experimental

In the successive fractions the group potency (anti-isohemoagglutinative) was measured to follow up the active factor as in the case of the Group **A** lipoid.

14 pancreases weighing from 40 to 105 g. (977 g. altogether) from individuals belonging to Group B were separately ground and dehydrated by standing with 4 volumes of denatured alcohol, followed by desiccation in a Faust apparatus. 127 g. of dry substance were thus given. It was Group **B**-active at a dilution of 1:2,000.

1st stage. The material was refluxed with two 650 cc. portions of denatured alcohol (99%), each time extending over 2 hours. The hot extracts (yellow-brown) were each quickly separated, while hot. They were combined and placed in an ice chest for 2 days, the occurring precipitate was filtered off and the filtrate was distilled in vacuo. The yellow-brown mucilaginous residue (5.7 g.; Group **B**-active at 1:50,000 dilution and Group **A**-active at 1:100) was washed with acetone and 3.0 g. of a yellowish, non-sticky, fine powder were given which was Group **B**-active at 1:100,000 and Group **A**-active at 1:100. It was fractioned with cold ether. The insoluble (2.5 g.) inhibited the isoagglutination of **B** erythrocytes even at a dilution of 1:200,000, and was further subdivided with cold chloroform. The portion remaining undissolved here (1.8 g., a yellow-brown, hygroscopic, hard clump) counteracted completely the

HEALTH CENTER LIBRARY

β-hemoagglutination at the same dilution as the ether-insoluble.

2nd stage. It was taken up in water and the solution was precipitated with lead acetate. The precipitate was suspended in water, bubbled with H_2S gas, and after addition of HCl to pH 2.0, centrifuged. The centrifugate was agitated with many changes of methanol, and the solutions distilled at low temperature to dryness. The still residue was again treated with methanol and the solution evaporated, washed with acetone and dried. The crop at this stage was 333 mg. It was perfectly inhibitory to the isoagglutination of **B** erythrocytes at a dilution of 1: 1,000,000.

3rd stage (*counter current distribution*). Solvent pair: an equilibrated mixture of water, methanol, benzene and toluene (1:4:4:1 by volume). 10 separatory funnels were used and 320 mg. of the product in the foregoing stage were distributed between 150 cc. of the upper phase and an equal volume of the lower phase in Funnel 0, and then the centrifuged upper layer was transferred to a second funnel. After the last transfer the contents of all the funnels were also shaken well, and from all the phases a 0.5 cc. portion was pipetted out and evaporated. The remainders were separately suspended in 0.5 cc. of physiological saline for the assay of the inhibition of the isoagglutination of **B** erythrocytes, which resulted:

	Funnel number									
	0	1	2	3	4	5	6	7	8	9
Upper phase	++	++	++	+	+	+	+	−	−	−
Lower phase	+++	+++	++	++	++	++	++	++	++	−

Control without any of the substances +++

−: no agglutination, +: slight aggl., ++: marked aggl., +++: strong aggl.

The A serum had agglutinin titre 80 and was diluted fivefold.

The upper phase of Funnels 7, 8 and 9 in which most of the Group **B** factor had accumulated were distilled together in vacuo to dryness. The residue was taken up in water by slight warming (40—50°C, 5 minutes) and after rejecting a trace of an insoluble part, redistilled and washed with acetone. 120 mg. of a fine powder with yellowish hue were given.

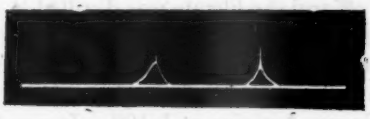


Fig. 1. An electrophoresis pattern of a 3/4% solution (opalescent) of the Group **B** lipoid preparation in phosphate buffer, pH 7.8, I 0.29; temperature 5°C; 10 ma. Exposure 50 minutes after starting current; mobility 8.6 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$. Ascending limb to the left.

It inhibited perfectly the isoagglutination of **B** erythrocytes at a dilution of 1:100,000. Elson & Morgan (tested after acid hydrolysis) and Molisch reaction positive. Biuret, Goldschmiedt, Neuberg & Saneyoshi and Rosenthaler negative.

Electrophoresis. The pattern in Fig. 1 was obtained with its solution in phosphate buffer of pH 7.8, which demonstrates only one boundary.

Regarding the quantitative composition, see Table I. The figures of the present product coincide fairly well with those of the one from pancreases of Group **A** persons.

The solubilities are listed in Table II.

TABLE I

Composition of the Group **B** Lipoid-preparation (Human Pancreases)

Analysis	Per cent
N*	2.4
P†	2.8
Reduction on ferricyanide of the hydrolysate as glucose‡	18.4
Hexosamine§	4.8
Ash‖	5.7

* Micro Kjeldahl. † Haneda, Sinokawa and Yasuoka's modification of Plimmer method. ‡ Masamune and Tanabe method. The hydrolysate (filtrate) contained none precipitable by phosphotungstic acid, so that it was immediately neutralized. § Hamasato-Akakura method.[2] ‖ Micro Pregl without use of H_2SO_4.

TABLE II

Solubilities of the Group **B** Lipoid-preparation (Human Pancreases)

−: insoluble, ±: faintly sol., +: partly sol., ++: mostly sol.

Solvent	Water		Methanol		Ethanol		Glacial acetic acid		Ether		Petrol ether		Benzene		Chloroform		Tetralin	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Solubility	+*	++	±	+	±	±	−	±	−	−	−	−	+†	+	±†	±	±†	++

* The solution was opalescent. † The undissolved particles swelled up and took a jellylike state.

The Education Department gave us a grant through the Grant Committee for Scientific Researches, which aided this work. H. Masamune.

References

1) Tokura, Tohoku J. Exp. Med., this volume, p. 299.
2) Hamasato and Akakura, J. Bioch., 1941, **34**, 159.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Effect of Piqûre diabétique upon the Blood Sugar Content of Rabbits, Adrenalectomized or Splanchnicotomized

By

M. C. Li

(李 茂 之)

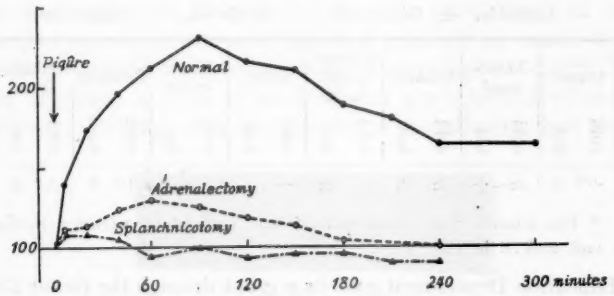
(From the Physiological Laboratory of Prof. Y. Satake, Tohoku University, Sendai)

(Received for publication, September 2, 1952)

The present research (1937) is a repetition of the work of Hatano from this laboratory (Tohoku J., 1936, **29**, 455). This was carried out in fact to try once more whether or not we would obtain by any chance a similar result with Stewart and Rogoff who were able to find commonly in adrenalectomized rabbits the hyperglycemia of the same scale as in control ones.

While Hatano removed the adrenal medulla, the splanchnic nerves were cut here. And glucose was given to rabbits, except 3 normal ones, in a dose of 5 g. for 7—10 days in addition to the routine food. In each set of experiments 8 individuals were experimented on. The liver was found to contain glycogen at the end of experiment in such amount: On an average 1.5 g. in toto & 0.8‰ of body weight in 4 control rabbits (only the puncture was done, glycogen was not determined in 4 remainings.), 1.2 g. & 0.6 ‰ in the adrenalectomized, and 1.4 g. & 0.65‰ in the splanchnicotomized.

The data rê the blood sugar are expressed in the accompaning figure; it tells by itself.



The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Rotatory Crossgraphy (Rotatory Cross Section Radiography) of Head

(Study on Rotatography, 7th Report)

By

Shinji Takahashi
(高橋 信次)

(From the Department of Radiology, Hirosaki University School of Medicine, Hirosaki)

and

Junnosuke Obara
(小原 準之輔)

(From the Department of Radiology, Tohoku University School of Medicine, Sendai)

(Received for publication, April 15, 1952)

Technique

The apparatus as well as the operating technique employed for the present study of rotatory crossgraphy[1),2),5),6)] was the same as were described in the preceeding reports.[3),4)]

Results

32 adult healthy persons were roentgenographed at eight cross sections of the head. Each of the sections was selected so as to be in parallel to the base cross section which contained angulus oculi temporalis and porus acusticus externus. They are called in the order from the top to the base the first, the second.....and the eighth section.

Topographicals in the x-ray image of those cross sections are as follows.

First Cross Section (Figure, not given):

This was radiographed at the height of the upper forehead.

The contour of the skull, oval or ellipsoid in shape, is surrounded by a similarshaped wide soft tissue. This bony contour is formed anteriorly by os frontale with projection of crista frontalis directed inwards, laterally by os parietale and posteriorly by os occipitale.

Second Cross Section (Figs. 1 and 2)

This was radiographed at the middle of the forehead.

The x-ray image of this cross section is similar to that of the first,

Fig. 1

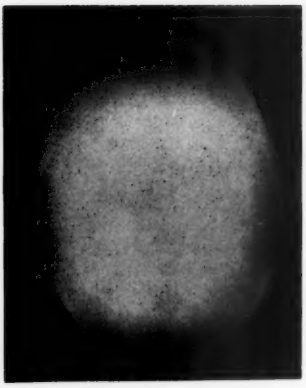
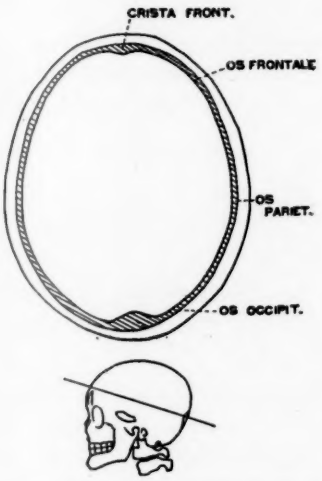


Fig. 2

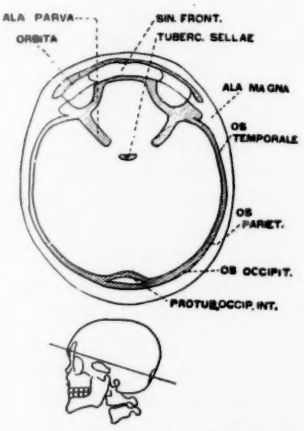


except for the occurrence of protuberantia occipitalis interna of os occipitale.

Third Cross Section (Figs. 3 and 4)

Fig. 3

Fig. 4



This was radiographed at the upper margin of the orbit.

In this cross section sinus frontalis is imaged at the most frontal part of the bony contour. The orbit is bounded posteriorly and laterally by ala parva ossis sphenoidalis, and mesially by the wall of sinus

ethmoidei. Approximately at the center of the bony contour, which is formed anteriorly by os frontale, laterally by ala magna ossis sphenoidalis, os temporale and os parietale, and posteriorly by os occipitale with the projection of protuberantia occ. int, is situated an x-ray image of sella turcica.

Fourth Cross Section (Figs. 5 and 6)

Fig. 5

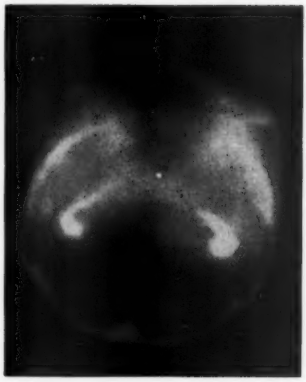
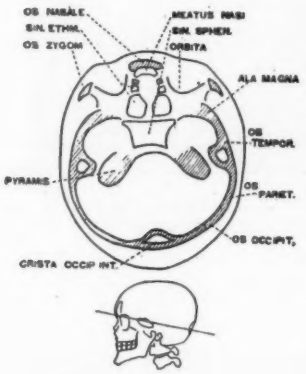


Fig. 6



This was radiographed at the middle level between the upper margin of the orbit and the base plane.

In this cross section cellulae ethmoidei is most remarkable. This sinus is bounded anteriorly by meatus nasi, situated backwards of os nasale, laterally by the orbit and posteriorly by ala manga ossis sphenoidalis. This sinus is imaged distinctly multilocular. A single image of sinus sphenoideus in large deformed contour is situated apart directly at the back of sinus ethmoidei.

The bony contour is similar in form to that of the third cross section. At about half way from the front, the upper part of pyramis is traversed.

Fifth Cross Section i. e. the Base (Figs. 7 and 8)

The contour of soft tissue increases in its thickness at the occipital area.

In this cross section sinus ethmoidei, sinus sphenoideus and auriculus interna are noticeable.

The position as well as the shape of these sini is similar to that in the fourth section except for the increase in its size.

At the back of fossa cranii media, situated laterally of sinus sphenoideus, is traversed pyramis. Accordingly, meatus acusticus externus and canalis caroticus internus, housed in the body of pyramis, are traversed

Fig. 7

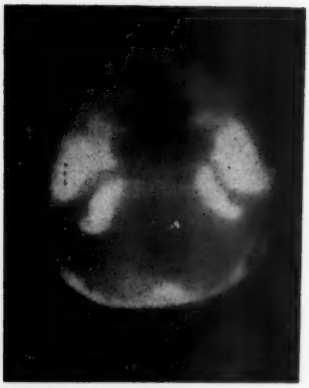
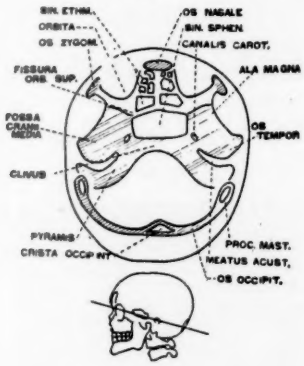


Fig. 8

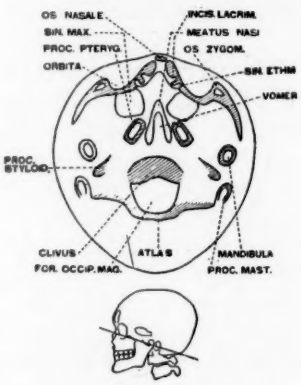


together, which reveals their relative positions along a straight line. This line, running laterally from mesial, is intersected by meatus acusticus internus. Clivus is imaged posteriorly to sinus sphenoideus.

Sixth Cross Section (Figs. 9 and 10)

Fig. 9

Fig. 10



This was radiographed at the lower margin of the orbit.

In this cross section maxillary sinus is noticeable. The cross section of this sinus is quadrilateral in shape and bounded anteriorly by the orbit, posteriorly by processus pterygoides.

The topographic relations between os nasale and the orbit are similar to those in the fifth cross section.

The latter, however, decreases in size, with two mesial concavities corresponding to sulcus lacrimalis at the front and cellulae ethmoidei

at the back. Meatus nasi posterior is triangular in shape, with an apex directed frontwards. By vomer this meatus is devided into two parts, the right one and the left.

Laterally to processus pterygoides are arranged in a line cross section of three bones, namely the body of mandibula, processus styloides and processus mastoides mentioned in order front to back. A large ring shaped image of foramen occipitale magnum is situated at the back of meatus nasi, with a contour formed laterally and posteriorly by condylus occipitalis.

Seventh Cross Section (Figs. 11 and 12)

Fig. 11

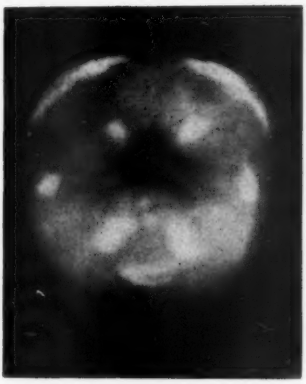
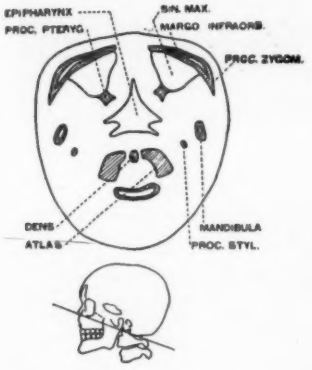


Fig. 12



This was radiographed at the level 5 mm lower than in the sixth one.

The cross section of maxillary sinus is imaged triangular in shape, with apeces directed backwards. This is bounded anteriorly by os zygomaticum and posteriorly by processus pterygoides. At the center of this cross section is imaged epipharynx with the projection of tours tubarius. The large ring at the back of epipharynx is the cross section of foramen vertebrae of atlas with dens of epistropheus.

Eighth Cross Section (Figs. 13 and 14)

This was radiographed at the level of meatus nasi externus.

At the front of the x-ray image mandibula with dentis is revealed in a shape of horse shoe.

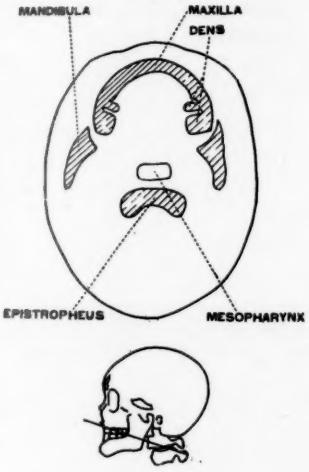
Pharynx, with a rise of the tonsil on either sides, is situated at the back of mandibula.

After studying the eight cross sections of the head with 32 cases we obtained the following results.

a) Shape of the skull in cross section

Fig. 13

Fig. 14



When inspected in the 1st and 2nd cross section, its contours of 23 cases out of 32 were symmetrical. Assymetrical cases of the rest were caused by the excessive projection of fossa occipitale, parietale, frontale either at the right or the left side. The shape in the cross sections of symmetrical cases was oval, ovoid or ellipsoid.

b) Thickness of the skull

The thickness of os parietale varied from 7 mm to 1 mm in life size. That of os frontale did from 7.5 mm to 2 mm, and that of os occipitale, from 9.6 mm to 3 mm.

c) Accessory nasal sinuses

The shape, size and developed abnormalities of sinuses of different normal cases vary so greatly that sinuses are never alike with one another. Sinus sphenoideus, for instance, were all asymmetrical and of the shape of a clover or glove. The average size counted 3.3 cm long and 1.84 cm broad. (The smallest in the base cross section was 1.0 cm long and 2.4 cm broad; and the largest, 5.6 cm long and 2.7 cm broad.)

Discussion

This study may be of use not only in the way of topographical orientation of organs or tissue in the living human body, but also in giving clinical, or operative informations to the surgeon. For instance, torus tubarius or illed cellulae ethmoidei posterior are difficult to inspect by the ordinary radiography.

References

1) Takahashi, S., Tohoku J. Exp. Med., 1950, **52**, 138.
2) Takahashi, S., Imaoka, M. and Shinozaki, T., ibid., 1951, **54**, 59.
3) Takahashi, S., Anzai, S. and Obara, J., ibid. in press.
4) Takahashi, S., and Obara, J., Nippon Acta Radiologica (in Japanese language) 1951, **11**, 24.
5) Gebauer, A., Fortschr. Roentgenstr. 1949, **71**, 667.
6) Vallebona, A., Estrotto da Scientia Medica Italiaca. 1950, **1**, 1.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Noradrenaline in Human Adrenals

By

Toshiyuki Ozaki
(尾崎 俊行)

(From the Department of Physiology, Nagasaki University School of Medie, cinNagasaki)

(Received for publication, September 10, 1952)

There are, as regards the noradrenaline content of human adrenals, only a few evidences (Euler[1]; Holtz & Schümann[2]; Shepherd & West[3]).

Recently I had a chance to estimate the noradrenaline contents of adrenal glands in a healty man of 30 years who died a violent death. The glands were extirpated about 19 hours after death and were extracted with 5% trichloracetic acid. For the determination of noradrenaline and adrenaline contents in these extracts, the permanganate method of Suzuki & Ozaki[4] was relied on. The results are tabulated.

TABLE I

Noradrenaline and Adrenaline Content of the Human Adrenals

Side of gland	Weight of gland g.	Adrenaline	Noradrenaline	Total	Noradrenaline
		mg. per g. of gland			%
R	4.0	0.934	0.130	1.064	12
L	3.5	0.958	0.110	1.068	10
	Mean	**0.946**	**0.120**	**1.066**	**11**

Noradrenaline was found to be present in an amount of 11% of the total in average, and this was in good accordance with the value of Shepherd & West (13% of the total). On the other hand, the estimates of adrenaline and noradrenaline contents (per g. of gland) of the present writer were about fourfold of those of S. & W.

References

1) v. Euler, U. S., Erg. Physiol., 1950, **46**, 301 f. 2) Holtz, P. Schümann, H. J., Nature, 1950, **165**, 683. 3) Shepherd, D. M. & West, G. B., Brit. J. Pharmacol., 1951, **6**, 665; J. Physiol., 1951, **114**, 25 P. 4) Suzuki, T. Ozaki, T., Tohoku J. Exp. Med., 1951, **54**, 332.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Macrocytic Anemia and Sato's Bromsulfalein Test in Children with Nutritional Dystrophy

(Studies on the Nutrition of Children in Hirosaki Area, 13th Report)

By

Yoshio Kakizaki and **Yuji Sato**
(柿崎 良男) (佐藤 雄治)

(From the Department of Pediatrics, Faculty of Medicine, Hirosaki University, Hirosaki; Director: Prof. Ts. Arakawa)

(Received for publication, April 19, 1952)

Macrocytic anemia is not uncommonly found among the children with nutritional dystrophy in Hirosaki Area.[1] Sato and Wada[2] demonstrated that macrocytic anemia of Type I (anemia with an increased volume index) was found in the majority of the children who were in a state of nutritional dystrophy and whose livers showed histopathological alterations revealed by needle biopsy specimens. On the other hand, Sato,[3] one of us, modified the bromsulfalein test for the purpose of detecting such histopathological changes in the liver as "cloudy swelling," which could not be revealed by means of the ordinary method of bromsulfalein test.

In the present paper we shall deal with macrocytic anemia of Type I among children with nutritional dystrophy, from the view point of "liver damage" estimated from results obtained by using Sato's bromsulfalein test.

Method and Materials

Blood examination[2] and Sato's bromsulfalein test[3] were carried out on school children who were apparently healthy but showed, on close examination, clinical manifestations of nutritional dystrophy to some extent.

The children, who gave positive results to the ordinary bromsulfalein test,[3] were excluded from the subjects to be investigated.

Results and Comment

As will be seen in Tables I and II, macrocytic anemia of Type I was found in 12 out of 22 cases giving positive results to Sato's test, while in none out of 11 negative cases.

TABLE I

Blood Pictures of Children with Nutritional Dystrophy, The Positive Cases by Sato's Bromsulfalein Test

No. of case	Name sex and age (y.m.)	Reds count (millions)	Hb (g.%)	Hemato-crit (%)	Volume index	Mean corpus-cular volume (μ^3)	White cell count
1	S.S. ♀ 6.7.	4.73	14	41	0.39	86	9800
2	N.S. ♀ 7.3.	3.49	12	41	1.29*	117	12000
3	K.M. ♀ 8.5.	4.78	13.5	41	0.93	85	10000
4	K.K. ♂ 11.1.	3.87	11	41	1.14*	105	10000
5	A.S. ♂ 11.1.	3.85	14	41	1.12*	106	7200
6	T.E. ♂ 10.7.	3.63	14	38	1.16*	104	8000
7	K.H. ♂ 11.6.	4.46	12	41	0.99	91	5000
8	A.S. ♂ 9.0.	3.81	13	44	1.26*	115	8800
9	J.S. ♀ 9.3.	4.26	14	39	0.99	91	9400
10	I.K. ♀ 9.11.	4.23	10.5	43	1.10*	101	10600
11	K.T. ♂ 12.4.	3.95	13	40	1.09	101	8400
12	I.B. ♂ 7.2.	4.05	12.5	39	1.05	96	9400
13	T.H. ♂ 7.5.	4.18	15.5	43	1.13*	102	8800
14	T.S. ♀ 9.3.	3.86	15.5	41	1.12*	106	15000
15	T.K. ♂ 10.4.	3.79	12	35	1.00	92	8800
16	K.Y. ♀ 11.11.	3.63	11	42	1.26*	110	6300
17	S.H. ♂ 10.8.	3.38	14	38	1.21*	112	6200
18	K.K. ♂ 12.0.	4.40	14.5	39	0.95	88	6200
19	K.S. ♀ 11.1.	4.23	11	40	1.02	94	9000
20	O.S. ♂ 11.3.	4.37	14	39	1.00	89	6400
21	O.H. ♂ 10.8.	2.70	15	39	1.57*	114	7000
22	W.F. ♂ 11.3.	3.38	14	37	1.12*	109	7200

* Macrocytic anemia: Volume index>1.10

In the preceding paper Sato and Wada[2] reported that macrocytic anemia of Type I was more frequently found in the cases with the liver of "cloudy swelling" than in those with the liver of diffuse fibrosis or of fatty infiltration, and they suggested, therefore, that, if folic acid plays a role in pathogenesis of macrocytic anemia of Type I, utilization of folic acid would be considerably disturbed in the livers of "cloudy swelling" revealed by biopsy specimens.

Sato's bromsulfalein test gives a positive result even when the histopathological changes in the liver are so slight in degree, that it may be impossible to detect them by the ordinary bromsulfalein test.

The results obtained from the present investigations demonstrate

TABLE II

Blood Pictures of Children with Nutritional Dystrophy, The Negative Cases by Sato's Bromsulfalein Test

No. of cases	Name sex and age (y.m.)	Reds count (millions)	Hb (g.%)	Hemato-crit (%)	Volume index	Mean corpus-cular volume (μ^3)	White cell count
1	N.H. ♂ 7.1.	4.94	11.5	38	0.84	76	
2	N.H. ♂ 6.11.	4.65	11	39	0.92	83	10800
3	O.K. ♂ 8.4.	4.56	10.5	38	0.90	83	7200
4	S.I. ♂ 8.2.	4.39	12	41	1.01	93	7800
5	K.T. ♂ 8.0.	4.84	11	40	0.89	82	8000
6	K.U. ♂ 8.3.	4.75	11	37	0.93	78	5600
7	I.T. ♂ 8.2.	4.83	11.5	38	0.85	78	21000
8	K.T. ♂ 10.3.	5.21	11	40	0.83	95	8800
9	O.H. ♂ 9.6.	4.02	11	36	0.97	89	7000
10	O.J. ♂ 10.5.	4.01	11.5	38	1.03	92	5200
11	K.N. ♂ 11.2.	4.36	11.5	39	0.96	89	6000

that macrocytic anemia of Type I is found only among children who are in a state of nutritional dystrophy and in whom the existence of certain histopathological alterations in the liver will be indicated by Sato's bromsulfalein test.

SUMMARY AND CONCLUSIONS

Sato's bromsulfalein test and blood examination were carried out on 33 school children who were in a state of nutritional dystrophy and from whom were excluded those who reacted positively to the ordinary bromsulfalein test.

The histopathological lesions in the liver in such a slight degree as revealed only by Sato's bromsuflalein test may be considered as one of factors contributing to the development of macrocytic anemia of Type I among children with nutritional dystrophy.

This work was carried out by a Grant in aid for developmental scientific research of the Ministry of Education. Prof. Ts. Arakawa.

References

1) Arakawa, Ts., Wada, Sh. and Takahashi, F., Tohoku J. Exp. Med., 1951, **55**, 61.

2) Sato, Y. and Wada, Sh., Macrocytic Anemia and Its Relation to Liver Biopsy of Nutrional Dystrophy in Children. ibid., in press.

3) Sato, Y., A Modification of Bromsulfalein Test and Its Relation to Liver Biopsy Findings in Children. ibid., in press.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Negative Phosphatase Reaction of Mitotic Chromosomes, Preliminary Note

By

Tom Mori
(森　　富)

(From the Anatomical Laboratory of Prof. M. Yamasaki, Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, September 18, 1952)

In rabbit bone marrow cells, negative phosphatase reactions of mitotic chromosomes in both acid and alkaline ranges were found, which formed a striking contrast with dark cytoplasm, while the resting nuclei were stained generally deeper than the cytoplasm. To check artificial errors, unspecific adsorption of calcium phosphate was tested according to Danielli,[1] incubating the inactivated slides in Gomori's alkaline mixture, which contained a small amount of hydrogen peroxide. As the impregnation of chromosomes took place synchroneously with that of the nuclei in this test, the negative reaction described above may represent a lack of alkaline phosphatase activity in the chromosomes. Adsorption of lead phosphate was examined according to Gomori,[2] putting together inactivated slide and non-inactivated one face to face, and incubating these pairs in Rabinovitch's[3] mixture. In inactivated slides, only the chromatin and chromosomes were impregnated. In the other slides of the pairs, the active ones, positive reaction which locates in the nuclear sap and cytoplasm was shifted into chromatin and chromosomes after long period of incubation. Accordingly, negative figures of the chromosomes in acid phosphatase reaction may be due to the natural absence of enzymatic activity in chromatin. Negative reactions of the chromosomes were also observed in the smears of sternal marrow obtained from two cases of myelogeneous leukemia. Detailed description will be given later in this Journal.

References

1) Danielli, J. F., J. Exp. Biol., 1945–46, **22**, 110; (Cited by Novikoff, A. B., Science 1951, **113**, 320).
2) Gomori, G., J. Lab. Clin. Med., 1950, **35**, 802.
3) Rabinovitch, M., Junqueira, L.C.U. and Mendes, F. T., Science, 1948, **107**, 322.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

A New Treatment for Gastric and Duodenal Ulcer

By

Seisyu Sakai

(酒井 清周)

(From the Medical Clinic of Prof. T. Kurokawa,
Tohoku University, Sendai)

(Received for publication, April 28, 1952)

Among many ways of treatment for gastric and duodenal ulcer one which has been adopted by physicians, native and foreign, is to protect the wound surface of ulcer from various stimulants, chemical or physical, by which cure is hastened. Mucin has been used for this purpose. I employed nagaimo, a kind of yam (Dioscorea Botatas, Decne), and had a remarkable effect. This makes me report here my discovery of a new treatment for the disease in the confident belief in its high effectuality.

Experimental

Preliminary Experiment

A yam is thoroughly washed, pared off, grated and then dried on a salver for 8~10 hours at a temperature of 37~38°C. It becomes wafer-like. This is ground in a mortar into powder. By this process 100 g. of grated yam gives 30~40 g. of powder. This powder is white, scentless, and somewhat moisture-absorbent, and tastes of arrowroot. By examining 0.1 g. of the powder in 10.0 cc. of distilled water about 6.0 of pH was given and 1.12~1.15 of viscosity by Hess's viscosimeter. After leaving at the room temperature for 30 minutes, 10.0 cc. of 0.5% hydrochloric acid, to which had been added 0.5g. of the powder, was titrated to find it to be neutralized with 12.02 cc. of 0.1 *N* NaOH (on an average of 5 examinations). These were conducted by the aid of Töpfer-reagent. When powder was not added to the acid in the same condition, neutralization required 13.72 cc. of 0.1 *N* NaOH. From these results it may be said that the corresponding amount of 0.5% hydrochloric acid with 1.70 cc. of 0.1 *N* NaOH combined with 0.5 g. of yam-powder. Another experiment was made with 0.25% hydrochloric acid. 0.5 g. of yam-powder combined with an amount of hydrochloric acid corresponding with 1.72 cc. (average of 5 tests) of 0.1 *N* NaOH.

This powder also showed its quality of combining with alkali, most

strongly with MgO_2, then $CaCO_3$, MgO, $NaHCO_3$ in a descending order.

I carried on pepsin determination through Fuld-Levinson's method and examined its ability of adsorption with pepsin. 1.0 g. of yam-powder was added to 10.0 cc. of filtrated gastric juice, which was placed in an incubator maintained at the temperature of 37°C for an hour. This was again filtrated, 2.0 cc. of which was employed for pepsin determination. The result was $P^{18°}_{30'}=4$; but if yam-powder was not added to the gastric juice, the result was $P^{18°}_{30'}=32$. This had made it clear that yam-powder not only neutralizes free hydrochloric acid, but adsorbes a considerable amount of pepsin.

Clinical Application

Materials and method. Materials for my experiment were 20 patients in our Clinic who had been correctly diagnosed as gastric or duodenal ulcer by X-ray and other clinical examinations. Among them 15 were patients of gastric ulcer, five of hyperacidity, of hypoacidity, and of normacidity, each. The other five were with duodenal ulcer. During the first three days they were made to take 2 g. of yam-powder, five times a day, and after the fourth day the same amount, three times a day. As a standard of testing the effect of the drug the following way was adopted. Subjective complaints were inquired every day, gastric analysis and X-ray examination were carried on almost once a week. Gastric juice was collected fractionally after Katsch and Kalk's method. Amounts of chlorine and pepsin were determined respectively by Rusznyak-method and Grützner-method. Under this treatment all other treatment was put aside and quiet rest and diet were ordered in the same condition as before treatment.

Results. Table I shows the days required for the recovery of subjective complaints of the patients. 11 cases out of 12 in which stomachache had been complained of became free from the pain in 1~7 (average 3.1) days, the other one, of ulcus penetrans, after 17 days. Hunger-pain disappeared in 2~8 (average 2.8) days, fullness of stomach in 4~11 (average 6.3) days, nausea in 1~3 (average 1.6) days, vomiting in 2 days, eructation in 3~12 (average 6.4) days, water-brash in 2~6 (average 3.8) days, pyrosis in 2~10 (average 5.1) days and anorexia disappeared in 2~9 (average 3.8) days.

Next I investigated the effect of the treatment on gastric analysis by dividing the examined cases into four groups from their acidity. The following description is as to the "after juice," as I conveniently call gastric juice after test meal had given. As for the averaged maximum values of their acidity I saved and tabulated them in Table II.

TABLE I

Days till Disappearance of Subjective Complaints

Symptoms	Cases	Days till disappearance	
		Min.~Max.	Average
Stomachache	1*	17	
	11	1 ~ 7	3.1
Hunger-pain	17	2 ~ 8	2.8
Fullness of Stomach	7	4 ~ 11	6.3
Nausea	5	1 ~ 3	1.6
Vomiting	1	2	2.0
Eructation	9	3 ~ 12	6.4
Water-brash	10	2 ~ 6	3.8
Pyrosis	11	2 ~ 10	5.1
Anorexia	7	2 ~ 9	3.8

* Ulcus ventriculi penetrans

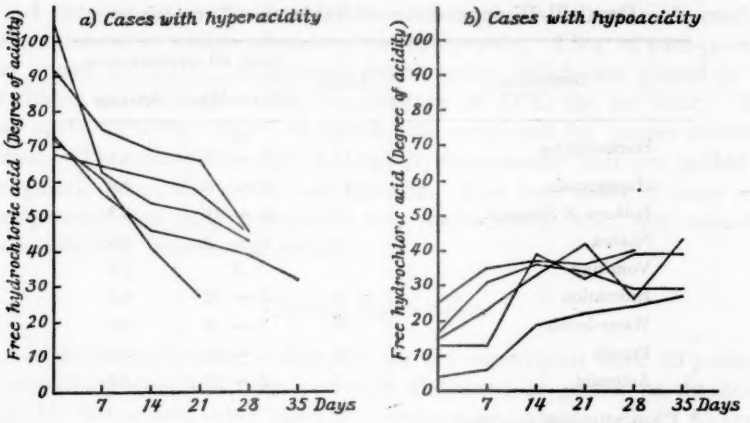
TABLE II

Average Value of the Highest Degree (before and after the treatment)

Group	Free hydrochloric acid		Total acid		Total chlorine		Neutral chlorine		Neutral chlorine / Total chlorine		Pepsin	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
I	82.0	40.4	39.0	51.6	502	421	259	349	0.51	0.83	640	640
II	15.1	32.5	26.5	45.2	369	399	324	213	0.87	0.53	320	640
III	38.7	34.5	55.4	59.9	419	401	306	307	0.73	0.76	640	640
IV	54.7	41.8	66.5	58.8	420	393	256	274	0.61	0.69	640	640

Group I. Cases of gastric ulcer with hyperacidity. As is shown in Fig. la, in all of the five cases, free hydrochloric acid decreased in concentration as days of treatment went on. Average reading 82.0° of the five cases before treatment was seen to drop to 40.4° after treatment. In parallel with free hydrochloric acid total acidity also decreased in all the cases, average reading falling from 93.0° to 51.6.° So is with total chlorine. Before treatment average reading was 502 mg. per dl, which came down to 421 mg. per dl after treatment. As is shown in Fig. 2a, average reading of neutral chlorine was 259 mg. per dl before treatment and 349 mg. per dl after treatment. The ratio of neutral chlorine to total chlorine increased from 0.51 to 0.83.

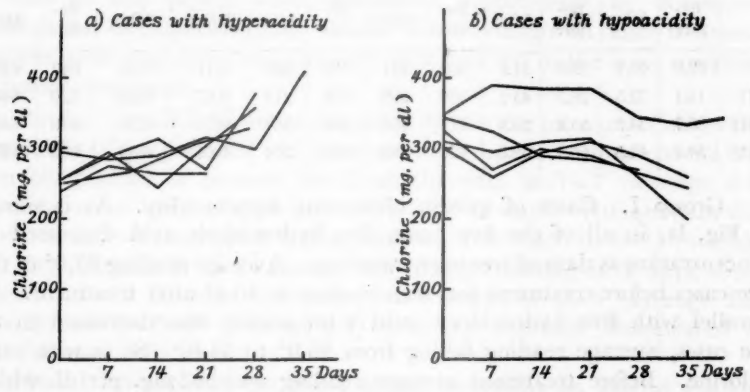
Fig. 1. Influence of yam-powder upon free hydrochloric acid.



Group II. Cases of gastric ulcer with hypoacidity. Fig. 1b shows the increase of concentration of free hydrochloric acid. Average reading 15.1° before treatment rose to 32.5° after it. Total acidity from 26.5° to 45.2.° Total chlorine from 369 to 399 mg. per dl.

Average reading of neutral chlorine fell from 324 to 213 mg. per dl (Fig. 2b). The ratio of it to total chlorine from 0.87 to 0.66.

Fig. 2. Influence of yam-powder upon neutral chlorine.



Group III. Cases of gastric ulcer with normacidity. As average value free hydrochloric acid and total acidity gave 38.7° and 55.4° before treatment and 34.5° and 59.9° after it, the former decreasing and the latter increasing a little. Average readings of total chlorine and neutral chlorine

were before treatment 419 and 306 mg. per dl respectively and after it 401 and 307 mg. per dl, there being no remarkable differences in either case. The ratios of neutral chlorine to total chlorine were on the average 0.73 and 0.76 before and after treatment.

Group IV. Cases of duodenal ulcer. Average readings of free hydrochloric acid before and after treatment were 54.7° and 41.8°; those of total acidity 66.5° and 58.8°; those of total chlorine 420 and 393 mg. per dl. Neutral chlorine increased from 256 to 274 mg. per dl in its average reading. The average ratio of neutral chlorine to total chlorine was 0.61 before the treatment and 0.69 after it.

In Groups I, III and IV, pepsin was observed in 1/640 (average maximum) dilute solution of gastric juice both before and after treatment, but in Group II, it was present in average 1/320 dilution of the juice before treatment and in 1/640 dilution after treatment.

Findings of fasting gastric juice were almost in parallel with the results of "after juice," but not always corresponding with them.

X-ray examination showed that niche disappeared in 4 weeks in 6 cases out of 15 with gastric ulcer, in 5 weeks in 4 cases, and in the other 5 cases it markedly diminished, though still present, when the patients left the Clinic. In the 5 cases of duodenal ulcer niche disappeared and the deformity of duodenal bulb was made strikingly slight, though not completely recovered, when the patients left our Clinic.

Summary and Discussion

I furnished yam-powder to 20 patients of gastric or duodenal ulcer and watched its effective progress. Subjective complaints were cleared almost within a week. Findings in gastric analysis were in almost all cases improved. That is, with the progress of treatment hyperacidity decreased and hypoacidity increased, both coming nearer towards normality. Though increase and decrease of total chlorine were not so marked as of acidity, it showed decrease in Group I and increase in Group II to some extent.

Considerable interest was claimed by the rise and fall in the neutral chlorine in each group shown with the progress of treatment. In all the cases of Group I it gradually increased as the treatment progressed, that is, the acidity decreased, while in Group II it tended to decrease as the acidity increased. Group III suffered a little change; Group IV showed a small increase.

Amount of pepsin, though it increased or decreased in individual cases, did not show any remarkable changes in its average value of each group between before and after treatment. A tendency of only a moderate

increase was shown in Group II.

Thus I observed findings of gastric analysis improved as treatment advanced together with subjective complaints. It was observed too by X-ray examinations that niche disappeared or diminished rapidly especially in gastric ulcer.

Next, in each case any remarkable side-actions were not experienced for about a month in which yam-powder had been being given, only in two or three cases a slight constipation was observed, which could be relieved by the aid of 0.5~0.7 g. of magnesii ustae.

The results mentioned above tell that yam-powder is of clinical use as an ulcer treatment. This yam is very easily obtained in this country throughout the year and its powder is also made through a simple method.

Now, what is the mechanism that brings about such a striking effect? The most desirable thing for a wound to be cured is that the part should be protected from all sorts of stimulants disturbing it. The surface of gastric or duodenal ulcer is always stimulated and damaged by gastric juice and diet. Moreover, the acidity of gastric juice of ulcer patients is said to be high in most cases in the acute stage. As a protector of ulcer surface from such stimulants Bucher[1] thought of gastric mucin. Fogelson[2][3] reports that with this mucin employed for clinical purpose he achieved a marked effect. He furnished 1 ounce of powder gastric mucin per day to 12 patients of gastric or duodenal ulcer and average 3 days administration made the symptoms greatly improved. He states that the reason is that the mucin not only protects the ucler surface but combines with free hydrochloric acid in gastic juice. Bradley[4] had testified experimentally that mucin obtained from gastric mucin of pigs inhibits the action of pepsin. In this country Fujioka and Yano[5] gave a report of remarkable efficacy of mucin against peptic ulcer. They tried mucin-therapy on 3 cases of gastric ulcer, 1 case of chronic gastritis, 2 cases of duodenal ulcer, 2 cases of gastric and duodenal ulcer, and 2 cases of post-operative complaints, and found that subjective complaints had disappeared in 2 or 3 days and objective symptoms such as bleeding, pain on pressure had also been rapidly relieved.

As mechanism of its action they have its protecting action of ulcer surface, combining ability with hydrochloric acid, inhibition of pepsin from digesting protein, etc., and further they think that it has an ability of counteracting the posionous action of cholic acid by combining with the acid in a stomach.

As to the components of yam which I employed for my experiment we have had Takahashi's[6][7] report. He explains that mucilaginous substance extracted from yam is similar to the mucins found in the animal world in that it has characters both of protein and of carbohydrate, but

is not of the same kind because it does not produce glucosamin through hydrolysis. Further he describes that this mucilaginous substance consists of a small amount of carbohydrate and a great deal of protein, the latter belonging to globulin and producing by hydrolysis arginine, histidine, lysine, cystine and tryptophan. It arouses our deep interest that the substance in question produces no histamine or histamine-like substance, but histidine etc. which have antagonistic acition to it.

Let us put together various facts given above and contemplate the mechanism of action of the yam-powder. It is clear that this powder contains mucilaginous substance and has, as has been said in the previous part of preliminary experiment, a combining ability with hydrochloric acid, as well as protecting action of ulcer surface. Further-more, when we acknowledge that there is less pepsin found in gastric juice with this powder added than in that without it, we may infer that this powder has an inhibiting action of the digestive ability of pepsin. These facts coincide very closely with the results of clinical application.

Conclusion

1. By furnishing yam-powder to patients their subjective complaints were relieved in earlier stages.

2. Gastric analysis showed that hyperacidity decreased and hypoacidity increased, both approaching normalcy, as the ulcer healed.

Neutral chlorine exhibited a tendency of gradual increase in cases of hyperacidity and gradual decrease in cases of hypoacidity, but in other cases it showed no marked variation.

3. Findings by X-ray examination proved there to be considerable and fast improvement in gastric ulcer, compared with which improvement in duodenal ulcer was slow.

4. No other side action than slight constipation was noticed.

5. Mechanism of action of yam-powder is to protect ulcer surface. But its combination with hydrochloric acid, inhibition of the ability of pepsin is thought to be contributory.

6. Yam-powder is found very efficacious in gastric and duodenal ulcer. It is easily to be had and taken, so its clinical value is believed very immense.

References

1) Bucher, R., Dtsch. Z. f. Chir., 1937, **236**, 515.
2) Fogelson, S. J. J., Amer. Med. Assoc., 1931, **96**, 673.
3) Fogelson, S. J., Arch. int. Med., 1935, **55**, 7.
4) Bradley, H. C., J. Biol. Chem., 1933, **100**, 20.
5) Fujioka, M. and Yano, R., Tokyo Iji Shinshi, 1936, No. 3005, 3081.
6) Takahashi, T., Kagami Kenkyu Hokoku, 1932, **21**, 1.
7) Takahashi, T., ibid., 1938, **45**, 1.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

"Peroxidase Response" of Erythron in Anemia, Preliminary Report

By

Ototaka Higashi

(東　晉高)

(From the Pediatric Department, Medical Faculty, Tohoku University, Sendai. Directors: Prof. A. Sato* and Prof. T. Sano)

(Received for publication, October 10, 1952)

I devised a modification of Sato-Shoji's[1] counting chamber method of copper peroxidase reaction (for leucocytes)——a counting chamber peroxidase method for erythrocytes. Under the method, erythrocytes showed a various degree of the intensity of peroxidase reaction, never recognized by film method.

I applied this method to 53 cases of various kind of anemia and 81 non-anemic cases and obtained the results that the peroxidase picture of erythrocytes in non-anemic cases was rather monotonous or uniform and that almost all the reds were of weakly peroxidase-positive type with only a few number of blue granules at most in one cell, while, in anemic cases, on the contrary, the picture was greatly altered and the variety of intensity of peroxidase reaction of each erythrocyte became very remarkable. Striking was the occurrance of a large number of strongly peroxidase-positive erythrocytes with abundant blue granules.

Further investigation of 6 other anemic cases i.e. one case of iron deficiency anemia of a weanling, one case of idiopathic thrombocytopenic purpura, one case of erythroblastosis fetalis due to Rh incompatibility, one case of anemia due to intestinal hemorrhage, one case of pernicious anemia and one infantile case of megaloblastic anemia, which were observed under the serial examination of peroxidase picture of erythrocytes, showed that a certain regularity must exist in the relationship between the peroxidase activity of erythrocytes or/and erythroblasts on one hand and the hemopoietic activity of erythron on the other. In these cases, at an early stage of blood regeneration, the peroxidase activity of erythrocytes in blood or and erythroblasts in bone marrow was greatly stimulated, in other words, strongly peroxidase-positive erythrocytes or/and erythroblasts were developed in blood or/and in bone marrow. So I desire to suggest the designation of "Peroxidase Response" of erythron for this phenomenon——a critical increase of strongly peroxidase-positive erythron in blood or/and in bone marrow at an early stage of blood regeneration in anemic cases treated specifically. It is well known that the reticulocyte response is the very first sign of blood regeneration. But the "Peroxidase Response" of either erythrocytes in blood or erythroblasts in bone marrow can, it seems, occur at an earlier stage than the reticulocyte response, as will be seen from my own cases——one infantile case of megaloblastic anemia and one case of pernicious anemia; in the latter case peroxidase picture was observed under serial bone marrow punctures.

The alleged striking increase of strongly peroxidase-positive erythrocytes in anemic cases might be an expression of "Peroxidase Response" of erythron.

The details will be reported in the near future in this Journal.

References

1) Sato, A. and Shoji, K.; J. Lab. and Clin. Med. 1928, **13**, 1058.

* now Honorary Professor.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Studies on Brain Stem

II. Comparative Anatomical Study of the Facial Nucleus of Mammalia

By

Shigeaki Yamamoto
(山本 重亮)

(From the Anatomical Laboratory of Prof. H. Seto,
Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, May 10, 1952)

Introduction and Materials

Since the publication of the famous "Neurobiotaxis" theory in 1907 by Kappers,[1)2)] many biologists supported the theory, so that the theory seems to have become a fixed authority, giving a powerful footing to the ontogenetic and phylogenetic studies of nervous system. However, there are some students, surely small in number, who are against this theory, e.g., Toyofuku[3)] and Fuse[4)] among them. In a word, the neurobiotaxis theory will have it that the motor nerve elements in the central nervous system actively move over toward the strongest stimuli against themselves in its genetic evolution. But those who oppose the theory assert that the moving over is not active but passive.

Now, according to the neurobiotaxis theory, the facial nuclei are moved in the phylogenetic and ontogenetic evolution in the following manner. The facial nuclei, which are located on the cranio-dorsal side in the incipient stage of development, are drawn to the nuclei of taste by the stimuli originating in those nuclei, and move backward from cranial to more caudal position. Then, following the development of the pyramidal tract, the strong stimuli originating therein cause the moving toward the pyramidal tract, that is, from dorsal to ventral side. Thus, the facial nuclei, first located in cranio-dorsal position move to ventro-caudal position. This is a very interesting theory, which I wished to verify in my study reported hereunder.

It is ideal to study the phenomenon both ontogenetically and phylogenetically to verify the theory, but in this report I will give my attention to the study from the phylogenetic standpoint, postponing my ontogenetic observations to my next report, Study on Brain Stem III.

For the materials of this study, I took brains of some mammalia belonging to the families of monotremata, edentata, insectivora, chiroptera,

cetacea, ungulata, carnivora, primates and men, which are made up in a series of transverse section preparations stained by Pal-carmine.

My Views

The relative positions of the nuclei facialis and fasciculi solitarii are not much diversified in all the above families of mammalia. In general, in making successive observation of the series of slides from the caudal side to the cranial, it may be seen that the facial nucleus begin to make appearance on the ventral side while the solitary nucleus is yet visible, though in wane. The latter disappears altogether subsequently while the former increases in size. In a few cases, the solitary nucleus disappears before the facial nucleus begins to become visible. However it may be, it is a general rule that the facial nucleus begins the existence at a level not far from the cranial end of the solitary nucleus, which pervades all families of the mammalia. In the relative development of the solitary nucleus there is hardly any difference observable between the families. Consequently, it is difficult to judge whether the facial nucleus has been moved to the position by the pull exerted by the solitary nucleus, since there is scarcely no difference in both the relative position and the relative development of both the nuclei.

So, in the following, I will speak with some length on the correlation of the pyramidal tract and the facial nucleus, without giving details on the relation of the solitary and facial nuclei. I will rather dwell on the secondary transfer of the facial nucleus, that is, the movement thereof from the dorsal to the ventral side, by the traction of the pyramidal tract.

The location of human facial nucleus is found in a transverse section in the vicinity of the ventral border of the medulla oblongata and the pons, immediately touching the dorsal side of the corpus trapezoides, the edge of the nucleus approaching the ventral border where the facial nucleus is developed at the strongest.

It is also well known that the pyramidal tract is well-developed in man. In describing my observations on many kinds of mammalia hereunder, I will take the position of the facial nucleus and the development of the pyramidal tract of man as standard of comparison. And then, the development of the pyramidal tract will be indicated by the ratio of the area of the pyramidal tract in cross-sectional area of the brain given in round numbers by rough estimate, without going into exact measurements of the area.

Monotremata

Echidna. These have a very poorly developed pyramidal tract, so that the tract seems to be forming a part of the lemniscus medialis on the

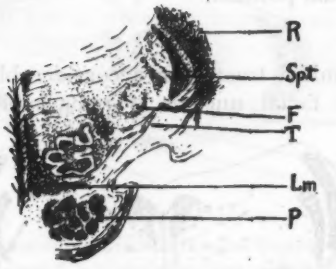


Fig. 1. Transversal section of brain stem in human adult, Pal-carmine staining, low power view. F facial nucleus; T corpus trapezoides; P pyramidal tract; Spt spinal tract of trigeminal nerve; Lm lemniscus medialis; R corpus restiforme.

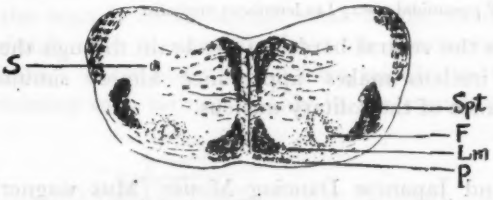


Fig. 2. Transversal section of brain stem in echidna, Pal-carmine staining, low power view. Poorly developed pyramidal tract P seems to be forming a part of lemniscus medialis Lm. F facial nucleus; Spt spinal tract of trigeminal nerve; S solitary nucleus.

dorsal side, to which it is almost fused in one in outer appearance. On the other hand, the facial nucleus is situated in a position similar to man and the development thereof is not at all weak. According to the neurobiotaxis theory, the facial nucleus should have remained in a more dorsal position, seeing the poor development of the pyramidal tract. The facial nucleus becomes visible at a level where the solitary nucleus is yet in sight.

Edentata

Ant-eater. The development of pyramidal tract is mediocre and the position of the facial nucleus is also normal, but rather nearer to the ventral border of the brain than in man. What is interesting in this species is the fact that the genu of the intrapontine facial nerve comes into view at a level where the facial nucleus has nearly disappeared, which shows that the pars I goes much farther toward the cranial side than in man.

Insectivora

Mole. The pyramidal tract is weakly developed, but the facial

nucleus is in the normal position.

Chiroptera

Bat. The pyramidal tract is in a considerably poor development. In comparison, the facial nucleus is remarkably well-developed. It

Fig. 3. Transversal section of brain stem in bat, Pal-carmine staining, low power view. F facial nucleus; P pyramidal tract; Lm lemniscus medialis.

almost touches the ventral border of the brain through the corpus trapezoides. The nucleus makes appearance almost simultaneously with the disappearance of the solitary nucleus.

Rodentia

Rabbit and Japanese Dancing Mouse (Mus wagneri rotans). In rabbits, the pyramidal tract is ill-developed and comes to end in the

Fig. 4. Transversal section of brain stem in rabbit, Pal-carmine staining, low power view. P Poorly developed pyramidal tract; F well developed facial nucleus; Lm lemniscus medialis.

cervical segments in the spinal cord, but in rats, another genus of the same family, the tract is strongly developed and continues down to the caudal part of the spinal cord. In the japanese dancing mice, a species of rats, the tract is not so strong in development, but yet somewhat better developed than in rabbits. However, the facial nucleus is extremely well-developed in both genera and almost comes into direct contact with the ventral border of the brain.

Cetacea

Dike Whale. The development of pyramidal tract is very poor in

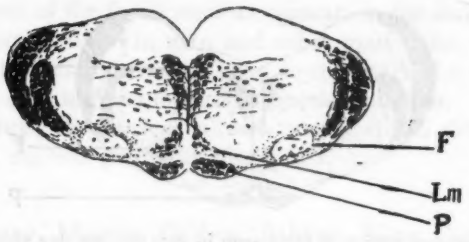


Fig. 5. Transversal section of brain stem in japanese dancing mouse, Pal-carmine staining, low power view. Facial nucleus F is almost come into contact with ventral border of brain stem. P pyramidal tract; Lm lemniscus medialis.

comparison with the total size of the brain. In the medulla oblongata, some meagre fibres are seen dispersed regularly without forming a bundle. In the pons, the tract is conspicuously weak in the strongly developed pons itself, existing only in one thin layer on the ventral side of the lemniscus medialis. These fibres do not form bundles in the caudal part of the pons, showing such formation only in the cranial part thereof.

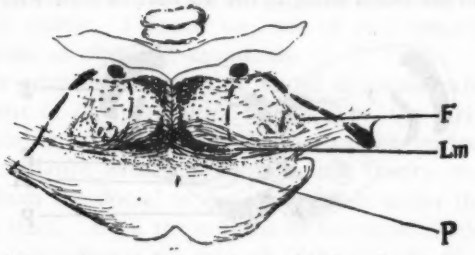


Fig. 6. Transversal section of brain stem in dike whale, Pal-carmie staining, low power view. Poorly developed pyramidal tract P dispersed without forming a bundle. F facial nucleus; Lm lemniscus medialis.

On the other hand, the facial nucleus is seen strongly developed at the level where the solitary nucleus remains in sight, developing further as the cranial part is reached. The position is much more on the ventral side than in man.

Ungulata

Horse and Goat (On Fourth Day after Birth). In goats, the pyramidal tract is particularly poor, disappearing already in the cranial part of the cervical segments of the spinal cord, but the facial nucleus cannot be said to lie in the dorsal side throughout its whole length of the nucleus.

The pyramidal tract of the horse is in average development, but its facial nucleus is situated in the normal location altogether, without any

Fig. 7. Transversal section of brain stem in goat (on 4th day after birth), Pal-carmine staining, low power view. P pyramidal tract; F facial nucleus; S solitary nucleus.

divergence from the normal. In both animals, the facial nucleus becomes visible while the solitary nucleus is still clearly observable.

Carnivora

Cat, Dog, Black Bear and Fur Seal. In dogs, bears and seals, the pyramidal tract is considerably well developed. The facial nucleus is also in good development, becoming visible while yet the image of the solitary nucleus remains observable. Its position is in the vicinity of the ventral border of the brain stem, in the normal, or somewhat on the ventral

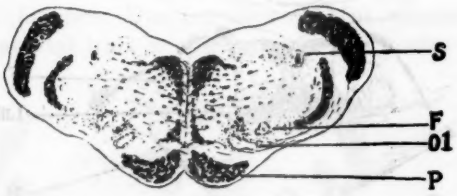


Fig. 8. Transversal section of brain stem in dog, Pal-carmine staining, low power view. P pyramidal tract; F facial nucleus; S solitary nucleus; 01 olivary nucleus.

Fig. 9. Transversal section of brain stem in seal, Pal-carmine staining, low power view. Nucleus olivaris metencephali Met is wrapped up in facial nucleus F. P pyramidal tract.

side, location. The case of the seal is of some interest. In the brain of a seal, unlike in other mammals, the facial nucleus becomes visible touching the ventral side of the nucleus ambiguus, while the latter is still in sight. The nucleus olivaris metencephali, which comes into sight on the level

of the cranial part of the facial nucleus, appears in the facial nucleus and wrapped up therein, while in man and other mammalia, this nucleus is seen adjoining the facial nucleus. Thus, in the brain of a seal is seen the interesting sight of a nucleus located in another nucleus. As the cranial end is approached, the nucleus olivaris metencephali develops further, while the facial nucleus gradually disappears.

Primates

Orang-Outang, Macaca, Gibbon, Babbon, Silver Gibbon, Siamang and Platyrrhini. In these animals, the development of the pyramidal tract and the relative position of the facial nucleus is nearly similar to that of human counterparts and nothing has to be mentioned in particular.

Summary

1. I undertook to make a comparative anatomical study of facial nucleus in many kinds of mammalia, at the same time making critical observation on the applicability of the neurobiotaxis theory by Kappers.

2. In making a serial study of the slides of brain tissue from the caudal up to the cranial side, the facial nucleus appears generally while the solitary nucleus is still visible. I found no case of any remarkable difference among the kinds of observed mammals.

3. In the observed animals, I found a considerable difference in the development of the pyramidal tract. Yet, the facial nucleus is in all cases invariably situated in the vicinity of the ventral border of the brain. In accordance with the neurobiotaxis theory, the facial nucleus should move from the dorsal to the ventral side under the stimulus from the pyramidal tract. If so, the position of the facial nucleus should show some difference according to the strength of the stimulus from the pyramidal tract, which strength in turn will be obviously determined by the development of the tract. But this is in contradiction to my observations described above. Consequently, the results of my study do not come in support of the neurobiotaxis theory.

References

1) Kappers, Neurol. Zentralbl., 1907, **26**, 834.
2) Kappers and Vogt, Neurol. Zentralbl., 1908, **27**, 958.
3) Toyofuku, Arb. neurol. Inst. Wiener Univ., 1910, **18**, 207.
4) Fuse, Neurol. Zentralbl., 1911, **30**, 178.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952.

Studies on Brain Stem

III. Ontogenetic Study on Translocation of Facial Nucleus

By

Shigeaki Yamamoto
(山本 重亮)

(From the Anatomical Laboratory of Prof. H. Seto,
Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, May 10, 1952)

In my previous report on the study of brain stem II appearing in this journal, I have given my observations on the movement of the facial nucleus in the early stage of development from the phylogenetic standpoint, at the same time proving the insupportability of the neurobiotaxis theory by Kappers.[1)2)] In this study I will study the same phenomenon ontogenetically, to assure better accuracy.

As material for the study, I took brains of human embryoes of 2, 3, 4, 5, 6, 7, 8, 9 and 10 months and new-born children of one and two week age, which are kept in our laboratory as a series of Pal-carmine stained transverse section slides.

My Views

In the brain of the 2nd month embryo, the differentiation of the intracerebral structure was extremely ill-developed and the nerve elements could be hardly distinguished.

In the 3rd month embryo, the differentiation of the nerve elements has developed to a stage where the nuclei of the cranial nerves could be clearly distinguished. At this stage, no myelination could be observed in any of the nerve elements, but the pyramidal tract could be seen as a bundle of extremely weakly developed fine fibres in the location where

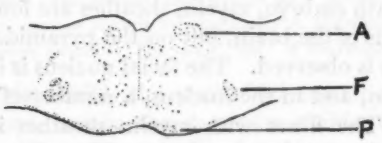


Fig. 1. Transversal section of brain stem in 3rd month human embryo, Pal-carmine staining, low power view. No myelination can be seen. F facial nucleus; A abducens nucleus; P pyramidal tract?

it should be. The facial nucleus is also bare of any myelin sheath but is clearly observable as a group of nerve cells, in the vicinity of the ventral border of the brain, that is, in a location quite similar to that of the nucleus in an adult brain. Its relative position to the solitary nucleus could not be ascertained, since the latter could not be observed with clarity.

In the 4th month embryo, no myelination was observable in the brain, but the pyramidal tract is far better developed beyond that in the 3rd month fetus. It is yet in poor development in the medulla oblongata, and also in the pons, though the pons itself is considerably developed.

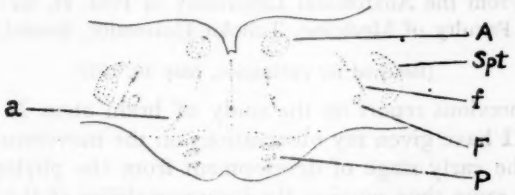


Fig. 2. Transversal section of brain stem in 4th month human embryo, Pal-carmine staining, low power view. No myelination can be seen. Pyramidal tract P is better developed than that of 3rd month embryo. F facial nucleus; A abducens nucleus; Spt spinal tract of trigeminal nerve; a abducens nerve; f intrapontine facial nerve (pars II).

The facial nucleus appears at a level where the solitary nucleus approaches the cranial end and indeed while yet the latter nucleus is in sight, in a study of the series of the slides starting from the caudal end up to the cranial. The former nucleus increases in size as the cranial end is approached. This relative position, as seen prevalent in my phylogenetic study, is invariable throughout the embryonic stages ontogenetically. The position of the facial nucleus is the same as that in the 3rd month embryo.

In the 5th month embryo, the pyramidal tract is larger in size, but no formation of myelin is yet observable. But in other nerve elements, some myelination has developed, and on the intrapontine facial nerve, a small number of very thin myelins are found. There is no change in the position of the facial nucleus.

In the 6th month embryo, myelin sheathes are found formed on most of the nerve elements of the brain, but on the pyramidal tract, not a trace of myelin formation is observed. The facial nucleus is in the same location as in the adult brain, and in the nucleus, a number of minute myelinated fibres are found. The fibres with myelin sheathes in the intrapontine facial nerve have increased in number.

In the 7th, 8th and 9th month embryoes, the nerve elements are seen to have greatly developed, both quantitatively and qualitatively, but

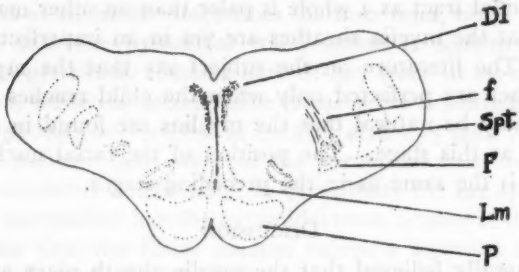


Fig. 3. Transversal section of brain stem in 5th month human embryo, Pal-carmine staining, low power view. Myelinated intrapontine facial nerve (pars I) f and dorsal longitudinal fasciculus Dl can be seen. P pyramidal tract; Lm lemniscus medialis; F facial nucleus; Spt spinal tract of trigeminal nerve.

nothing else deserves special mention.

In the 10th month embryo, at last some trace of myelination on the pyramidal tract can be detected, but the myelins are extremely few and very thin on the nerve fibres, and seemingly yet imperfect in quality, so that they stain ill and indistinct, and are observed as if light and minute dots are scattered over the tract. But the percentage of the pyramidal tract in the substance of the pons and the medulla oblongata is not lower in comparison with that of the adults. The facial nucleus shows itself as almost completely developed and the position is exactly the same as that of the adult brain.

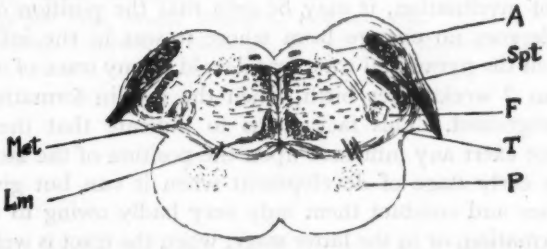


Fig. 4. Transversal section of brain stem in 10th month human embryo, Pal-carmine staining, low power view. On pyramial tract P some trace of myelination can be detected. F facial nucleus; Met nucleus olivaris metencephali; A abducens nucleus; T corpus trapezoides; Lm lemniscus medialis; Spt spinal tract of trigeminal nerve.

In the seven day child, the pyramidal tract had only a very weak myelination to show, but in the 2 week's child, the myelination has developed very rapidly. The myelin sheathes are observed all over the transverse section of the pyramidal tract and seem to have been formed on every nerve fibre. Yet the individual sheathes are thin and the stain

on the pyramidal tract as a whole is paler than on other nerve elements. It is clear that the myelin sheathes are yet in an imperfect stage of development. The literature on the subject say that the myelins on the pyramidal tract are perfected only when the child reaches two years of age, and it may be natural that the myelins are found in an imperfect development at this stage. The position of the facial nucleus in the 2 week's child is the same as in the preceding stages.

Discussion

It is generally believed that the myelin sheath plays an important, though may be not a decisive, part in assisting the conduction of impulses of nerve fibre. Consequently, when the myelin in a myelinated nerve fibre is imperfect, the function of the nerve fibre also cannot be perfect, that is, the function of a nerve fibre becomes perfect only when the formation of the myelin sheath is completed. According to Szymonowicz's[3] text-book, the excitability of a nerve is low by a new-born child, but the excitability grows with the progress of the myelination. In adults, the nerve fibres with speedy conduction of impulses are provided with thick myelin sheathes.

Now, in my ontogenetic study, I have ascertained that the position of the facial nucleus is always the same and invariable from the time when the pyramidal tract is in a quantitatively very poor stage of development in the early embryonic life to the last part of the embryonic life when the development of the tract is almost completed. From the view-point of myelination, it may be seen that the position of the facial nucleus undergoes no change from where it was in the infancy of the embryo, when the pyramidal tract was devoid of any trace of myelination, till as late as 2 weeks afrer birth, when the myelin formation has considerably progressed. This fact seems to indicate that the pyramidal tract does not exert any influence upon the position of the facial nucleus, either in its early stage of development when it can but give out only weak impulses and conduct them only very badly owing to the absence of myelin formation, or in the latter stage, when the tract is well developed and better myelinated, and surely gives out stronger impulses under better conduction. In short, it shows that the impulse from the pyramidal tract is not a cause effecting a change in the position of the facial nucleus. This is utterly in disagreement with the theory of neurobiotaxis according to which the facial nucleus should transfer its position nearer to the pyramidal tract attracted by the impulses originating in the tract. Accordingly, I am not in the position to support the neurobiotaxis theory as far as it concerns the subject of my study.

Furthermore, it is generally assumed that the larger nerve fibres

are myelinated at an earlier stage than the finer ones, but I noticed with some interest that the pyramidal tract, which is composed, generally speaking, of larger fibres, remains bare of any trace of myelins in a 5th month embryo, while the intrapontine facial nerve is provided with some myelins, though may be very few in number and very poor in size. This fact seems to indicate that, whereas the pyramidal tract and the facial nucleus are correlated functionally, in excitability the intrapontine facial nerve is more susceptible than the pyramidal tract, at least in the embryonic stage, and also that the facial nucleus enjoys a separate and inherent development independent of the influence of the pyramidal tract and an extent of independent function. I believe that the fact that the facial nucleus may have a function of its own furnishes another basis to the assumption that the facial nucleus is not necessarily translocated under the influence of the pyramidal tract, as the neurobiotaxis theory will have it.

Conclusion

I made an ontogenetic study of human infancy starting from a 2nd month embryo and ending with a two week child and ascertained that the facial nucleus takes up its position from the first, and is not displaced later on, as the tenet of the neurobiotaxis theory seems to indicate.

References

1) Kappers, Neurol. Zentralbl., 1907, **26**, 834.
2) Kappers and Vogt, Neurol. Zentralbl., 1908, **27**, 958.
3) Szymonowicz, Lehrbuch d. Histologie, 5. Aufl., Curt Kabitzsch Leipzig, 1924.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Electroencephalographic Study of Conditioned Salivary Reflexes in Human Subjects

By

Kituya Iwama and **Mituyosi Abe**
(岩間吉也) (阿部實賢)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai)

(Received for publication, May 15, 1952)

In experiments of successive conditioned reflexes, the procedure of conditioning is carried out in such a way that a conditioned stimulus is followed by an unconditioned stimulus at a considerable time interval. In this case, the conditioned reflex, when established, takes place with a delay almost equal to the time interval between the conditioned and the unconditioned stimulus. Such a response is called a delayed response.

However, the delayed response is not the only one which can be seen in the successive conditioned reflex. In his experiment on conditioned galvanic skin reflexes (GSR), Motokawa[1)2)] found that two GSRs appeared in succession upon the conditioned stimulus alone. The second response was obviously a delayed response, because it appeared with a considerable delay. It was, however, scarcely expected that besides the delayed response an additional one should appear soon after the onset of the conditioned stimulus. For simplicity, we shall designate such a response of a short latency as an initial response.

Because Motokawa used an acoustic stimulus as a conditioned stimulus, he was cautious of regarding the initial GSR as a conditioned reflex. Taking into account that the acoustic stimulus is most effective upon GSR in the psychic passive state of the subject, he regarded the initial response as an unconditioned response to the conditioned stimulus. His interpretation seems to be supported by the finding that at a more advanced stage of conditioning the initial GSR was gradually weakened while the delayed one increased in magnitude.

In their studies on conditioned GSRs in human subjects, Switzer[3)] and Rodnick[4)5)] found that an initial response appeared in company with a delayed response, but scant attention has been paid to the nervous mechanism underlying the initial response. In the present investigation, we worked out a salivary conditioned reflex in human subjects and found that the pattern of conditioned salivary reflexes was almost the same as

that of the conditioned GSR. Various aspects of the reflex were extensively studied with special attention to the initial response.

Recent experiments by Motokawa,[2] by Motokawa *et al*[1] and by Iwama[6] demonstrated that much information about the central processes involved in conditioned reflexes can be obtained from electroencephalograms (EEG); an excitatory process manifested itself in an electrical pattern consisting of usual excitatory waves (β-or θ-waves), while a well-augmented α-activity indicated a development of an inhibitory process. In the present study, the central mechanism of the salivary conditioned reflex was studied on the basis of electroencephalographic findings.

Experimental

Method

Throughout the present experiment, we took an electrical activity of the parotid as an index of the secretion reflex. As shown by Iwama and Shinjo[7] the slightest activity of the parotid can be recorded. By means of this method action currents of the parotid were recorded together with EEG.

Brain potentials were picked up by a monopolar leading with a different lead placed on the vertex and an earthed lead on the earlobe.

As the unconditioned stimulus we used solutions of acetic acid, ranging from 0.5 to 3 per cent in concentration. A sufficient amount of the solution was stored in a reservoir bottle from which a stimulus solution of about 5 c.c. was poured through a rubber tubing into the subject's mouth. The administration of the stimulus solution was carried out by the experimenter with the aid of an air-transmission apparatus. Metronome beats were used as the conditioned stimulus. They were so weak that the subject showed no startle reaction upon them.

In one series of experiment the subject was given the paired stimuli of the beat and the acid from five to ten times. Each administration was made at an interval of three to five minutes. In order to observe the effect of the conditioned stimulus alone, we sometimes applied the beat without the acid.

Results

1. *Activity pattern of the parotid and of the brain in a successive salivary conditioned reflex*

Fig. 1 illustrates the records taken at various stages of conditioning. In this experiment the metronome beat was applied for about 20 seconds and the reinforcement was made at about the 12th second from the beginning of the former. The well-established reflex pattern is presented in

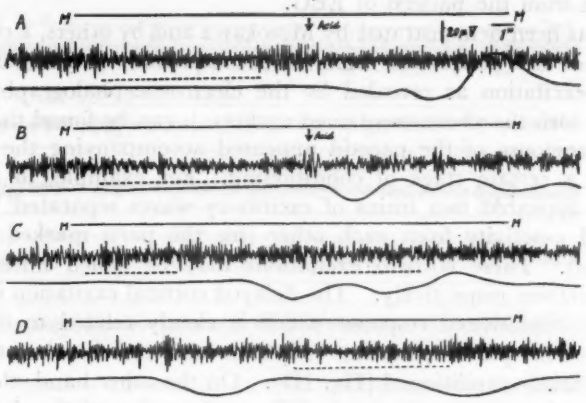


Fig. 1. Activity patterns of the brain and of the parotid in the course of establishment of a salivary conditioned reflex. Conditioning proceeded from A to D. In all records the line MM indicates the duration of the conditioned stimulus (metronome beat). A & B: Records in the early stages of conditioning. The unconditioned stimulus (acid solution) was given at about the 12th second from the onset of the metronome beat (the arrows). The elicited action currents of the parotid represent unconditioned responses. C: The conditioned stimulus alone could elicit a parotid response, but the complete conditioning was not yet attained. D: Record in a well-conditioned state. The initial and the delayed response of the parotid appeared well separate from each other.

As to EEG, the delayed conditioned excitatory waves could be found in C & D (see the parts marked by the dotted underlines). The initial excitation of the brain was very marked in A & B, but it became less conspicuous as the conditioning advanced (C & D).

Fig. 1D, where the action current of the parotid appeared two times successively during the application of the conditioned stimulus alone. There is no doubt that the preceding action current represents the initial response and the succeeding one the delayed response. As is the case in other kinds of successive conditioned reflex, the delayed response is a conditioned reflex which we aimed to establish, because its latency is almost the same as the time interval between the beginning of the conditioned stimulus and the reinforcement. Concerning the initial response we can claim that it is not an unconditioned response to the conditioned stimulus. Because, 1. the metronome beat is evidently neutral to the secretion reflex at least under the ordinary conditions of the subjects, and 2. the initial response begins to appear after the conditioning procedure has been applied several times.

Thus, we have come to a conclusion that the initial response of the parotid is no less a conditioned reflex than the delayed response. However, these two kinds of responses have different properties which can be

evidenced from the pattern of EEG.

As has been demonstrated by Motokawa and by others, a conditioned response of the peripheral organ is accompanied with a cortical conditioned excitation as revealed by the electroencephalography. In accordance with the above-mentioned authors, it can be found that the conditioned response of the parotid appeared accompanying the excitatory waves at a certain stage of conditioning. For example, in the record 1C there appeared two trains of excitatory waves separated by a well-developed α-activity from each other (see the parts marked by dotted underlines). These cortical excitations may be called initial and delayed excitation respectively. The delayed cortical excitation is evidently a cortical conditioned response which is closely related to the delayed response of the parotid, because it is seen most clearly when the parotid has been firmly conditioned (Fig. 1D). On the other hand, the behavior of the initial cortical excitation is different from that of the delayed excitation. As will be seen from the series of records presented in Fig. 1, the initial cortical excitation becomes less conspicuous as the conditioning advances and finally it is almost completely abolished in the well-conditioned state. Thus, it has been made clear that the delayed response of the parotid is accompanied with the cortical conditioned excitation while the initial response is not at least after the complete conditioning.

2. *Experiment on generalization and extinction*

With a term generalization we refer to the fact that, when a conditioned reflex has been established to one stimulus, another allied stimulus elicits qualitatively the same conditioned response without reinforcement (stage of generalization). If the allied stimulus is repeated without reinforcement, the generalized conditioned response is gradually decreased in magnitude and finally disappears. Thus the differentiation of the two stimuli is completed with respect to the conditioned reflex at issue (stage of differentiation). As exemplified by the records in Fig. 2, we found that in the salivary conditioned reflex studied by us the differentiation was established according to this general principle.

At a stage of generalization (Fig. 2B), the conditioned reflex was generalized to the unreinforced stimulus with respect to the initial as well as to the delayed response. After several repetitions of the unreinforced stimulus, a stage of differentiation was reached in which no generalized response whatever was elicited by the unreinforced stimulus while the reinforced stimulus was effective upon the parotid to give rise to the ordinary reflex activity (Fig. 2D & 2E).

The reflex pattern at the intermediate stage between the generalization and the differentiation deserves attention. At this stage the un-

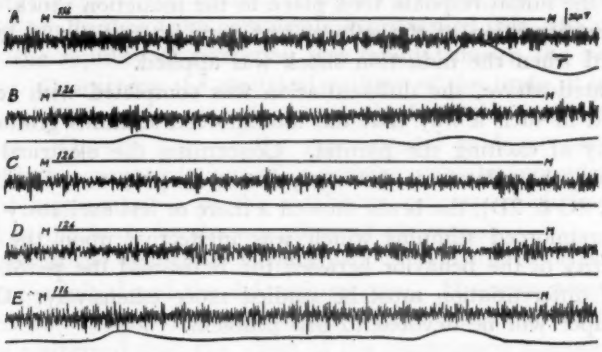


Fig. 2. Process of differentiation. A conditioned salivary reflex was established to the metronome beat of 116 times per minute (M_{116}). The stimulus to be differentiated from M_{116} was M_{126}. A: The effect of M_{116} in the well-conditioned state. The initial and the delayed response of the parotid could be clearly shown. B: The stage of generalization. The two responses of the parotid could be generalized to M_{126}. C: The stage of incomplete differentiation. Only the generalized initial response appeared upon M_{126}. D: The stage of complete differentiation. M_{126} could not excite the parotid. E: M_{116} was still capable of giving rise to a conditioned reflex of the parotid after the completion of the differentiation. Note that EEG in the course of differentiation (B, C & D) exhibited more or less excitatory waves.

reinforced stimulus could elicit the initial response but failed to develop the delayed one. It is evident from this result that there is a greater tendency for the initial response to be generalized than for the delayed response.

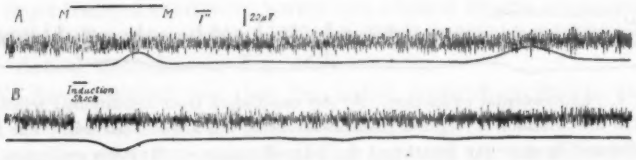


Fig. 3. Generalization of the initial parotid response upon an induction shock. A: An established trace conditioned reflex in which the metronome beat of 5 seconds in duration was used as a conditioned stimulus. B: An induction shock was applied to the subject's skin. A generalized initial reponse could be seen.

In an additional experiment it was found that the initial response could be generalized to a stimulus whose modality was quite different from that of the reinforced stimulus. As will be seen from the record 3B, an action current of the parotid appeared soon after the application of an induction shock to the subject's skin. It is obvious that the general-

ization of the initial response took place to the induction shock. In this case the introspection of the subject was very interesting; a sour taste was evoked when the induction shock was applied.

As stated above, the differentiation was completed with respect to the parotid in such a way that the unreinforced stimulus gradually lost its capacity of exciting the parotid. Concerning the electrical activity of the brain, the relation is, however, not so simple; as evidenced from EEG (Fig. 2C & 2D), the brain showed a more or less excitatory response to the unreinforced stimulus which was ineffective upon the parotid. The disparity of the behavior between the brain and the parotid in the process of differentiation must be studied more extensively. Our next coming paper will be devoted to this problem.

3. *Experimental extinction and disinhibition*

As is well known, the procedure of presenting a conditioned stimulus unaccompanied by a reinforcement causes a decrement in a conditioned response. We conducted such a procedure so as to make clear which of the two responses, initial or delayed, decreases more rapidly.

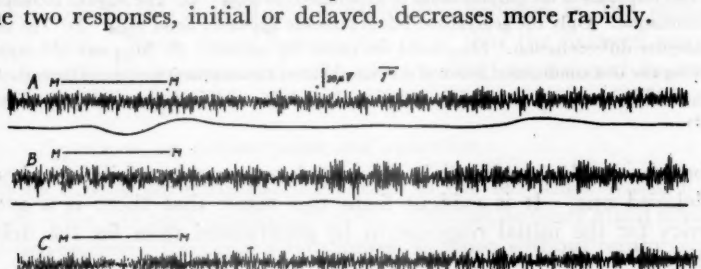


Fig. 4. Experimental extinction. A: An established trace conditioned reflex. The duration of the conditioned stimulus (metronome beat) was about 5 seconds. The parotid was conditioned to show the initial and the delayed response. B: After extinction no response of the parotid appeared. α-activities were very markedly augmented. Between B and C a disinhibiting procedure was applied in such a way that the subject was given a small amount of strong acid without previous notice. C: The conditioned stimulus could elicit the initial response of the parotid and the initial excitation of the brain. D: Repetition of the conditioning procedure could restore the original reflex pattern.

In the first place, it was found that the salivary conditioned reflex once established was very difficult to be extinguished. A complete extinction could be achieved only when a very dilute acid solution was used as an unconditioned stimulus. As the procedure of extinction was

repeated, the initial and delayed responses gradually decreased in magnitude and finally they were completely extinguished (Fig. 4B). No difference in the rapidity of extinction was found between the two kinds of responses. Concerning the electrical activity of the brain, it is noticeable that α-activities were markedly augmented at the stage of complete extinction. On the basis of the experimental evidence previously obtained,[1)6)] the augmentation of α-activity can be considered as an electrical representation of the inhibitory process which has been developed in the brain by the procedure of extinction.

So far as the extinction is concerned, there seems to be no difference between the initial and the delayed response. However, some difference was seen concerning disinhibition, as will be mentioned below. After we have confirmed that the effect of the conditioned stimulus upon the parotid has been completely abolished, we carried out a disinhibiting procedure by applying an extra-stimulus to the subject without previous notice. If the disinhibiting procedure applied is sufficient to remove the prevailing inhibition, the conditioned stimulus may be effective upon the parotid once again. It was found, however, that the effect of our disinhibiting procedure was insufficient to free the parotid completely from the inhibition. Because, when the conditioned stimulus alone was applied immediately after the disinhibiting procedure had been finished, the parotid showed only the initial response (Fig. 4C). The repetition of the usual procedure of reinforcement was required in order to bring about the delayed response once again.

It is to be noted that the activity of the brain after the application of the disinhibiting procedure was entirely different from that in the state of inhibition; the bursts of well developed α-activities could not be found and a short train of excitatory waves was elicited by the conditioned stimulus just corresponding to the initial parotid response (see the part indicated by the dotted underline in Fig. 4C).

We have pointed out that the initial cortical excitation can be observed only at the early stages of conditioning, and that the appearance of the excitatory waves ensued at the onset of the conditioned stimulus after disinhibition. In view of these facts it may be said that the state of the brain is brought back by a disinhibiting procedure to the initial state of conditioning.

Discussion

In our study on successive conditioned salivary reflexes, it has clearly been shown that the two separate activities of the parotid appear successively during the application of the conditioned stimulus alone. If the volume of the saliva secreted is taken as an index of the activity of

the parotid, it may be difficult to make such clear separation of the two successive responses. Because the saliva is secreted so slowly that we obtain only one confluent response when the parotid is excited in succession at a short time interval.

As stated in the introduction, the initial response was observed also in the conditioned GSR, but it has been unsettled whether or not the initial response in this case can be regarded as a conditioned reflex. We, therefore, conducted an experiment of conditioning a GSR so as to make clear the situation. As exemplified by the records in Fig. 5, we have proved that the initial GSR is a conditioned reflex in the same sense as is the delayed GSR. In our experiment on the conditioned GSR, the possibility has been ruled out that the initial response is an unconditioned

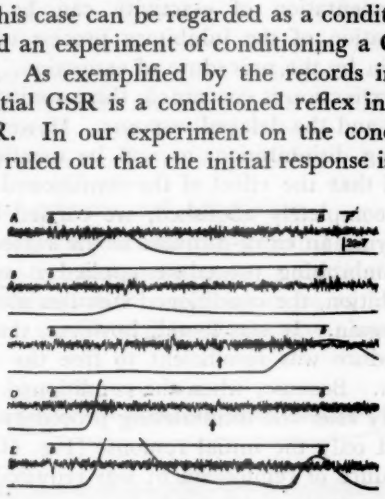


Fig. 5. Conditioning experiment of galvanic skin response (GSR). EEG and GSR were simultaneously recorded. The conditioned stimulus was a sound from an electric bell whose duration was indicated by the lines BB in all the records. The unconditioned stimulus was an induction shock applied to the subject's skin. A & B: Unconditioned GSRs to the sound. The magnitude of the unconditioned GSR was gradually decreased as the sound was repeated without being accompanied by the induction shock. C: The sound was followed by the induction shock (the arrow). An unconditioned GSR appeared upon the induction shock, but not upon the sound. D: The sound became effective upon GSR once again. The elicited GSR represents the initial response. E: The effect of the conditioned stimulus alone examined. The initial and the delayed GSR appeared well separate from each other.

response to the conditioned stimulus. Because our experiment was performed after the unconditioned GSR to the conditioned stimulus had been completely abolished by repeating the conditioned stimulus alone.

By studying the salivary conditioned reflex the conclusion has been easily arrived that the initial response is a conditioned reflex. In a conditioned reflex which is formed on the basis of the salivary reflex there is no reason to consider the initial response as an unconditioned response to the conditioned stimulus. Because it is evident that the unconditioned salivary reflex can be elicited usually by the gustatory stimulus.

The classical theory of the conditioned reflex[8] has been based upon the assumption that all the conditioned responses of the peripheral organ are controlled by the cortical activity. But this assumption must be slightly revised, because a number of investigators[9] demonstrated the possibilities of conditioning at a subcortical level. Therefore, it is likely that a conditioned reflex is executed by the subcortex as well as by the cortex. But we may infer that in some cases the activity of the cortex is more important than that of the subcortex, while the reverse is true in other cases. From the pattern of EEG we have pointed out that the initial response of the parotid is not associated with a marked cortical excitation at least in a well-conditioned state. This fact probably means that the cortex takes no important part in the initial parotid response. On the other hand, it has been observed that the conditioned excitatory waves in EEG preceded the delayed response of the parotid. It is likely that a prominent part is played by the cortex in the delayed response.

These assumptions seem to be supported by the finding that the initial parotid response has a greater tendency to generalization than does the delayed response, because it has been found that a conditioned reflex established in a decorticated animal can easily be generalized. In other words, a fine differentiation can be observed only in the conditioned reflex which is under the control of the cortical activity. Since the initial response seems to be independent of the cortical activity, it is quite natural that a generalized initial response can easily be obtained.

Now, attention should be called to the fact that there appears the initial excitation of the cortex upon the conditioned stimulus at the early stages of conditioning. Probably such an initial cortical excitation may be an unconditioned response of the cortex, because it is almost completely abolished in the well-conditioned state. However, it is noticeable that the conditioned stimulus which had been taken for almost ineffective could give rise to a fairly strong cortical excitation in the experiment of conditioning, and that at a certain stage of conditioning the initial parotid response could be related to the initial cortical excitation. From these considerations it is probable that the cortex makes some contributions to the execution of the initial response at least in the early stages of conditioning. Such being the case, we can not regard the initial cortical excitation as a mere unconditioned response to the conditioned stimulus. In this respect a more detailed study seems to be required.

Since the conditioned salivary reflex can be considered a preparatory reaction of the organism to the acid stimulus, the initial response of the parotid seems to be a superfluous reaction. From the standpoint of economics of brain energy it seems unlikely that the organism consumes a certain amount of energy for such a superfluous reaction. Therefore,

the nervous activity for the initial response must be more and more strongly suppressed and finally confined to a certain limited region of the brain. From this consideration it is understandable that the initial cortical excitation of the cortex becomes hardly accessible to the electroencephalography as the conditioning advances. If this kind of central excitation is suppressed to a certain extent, the related response of the peripheral organ ceases to appear. The complete extinguishment of the initial response has been reported by Motokawa *et al* in the conditioned GSR. To reach such a state of conditioning was found more difficult in our experiment on salivary conditioned reflex, but it could be realized when a very dilute acid was used as an unconditioned stimulus.

Summary

A salivary conditioned reflex was established in human subjects by applying a metronome beat (conditioned stimulus) and a small amount of acid solution (unconditioned stimulus) successively at an appropriate time interval. The action current of the parotid was taken as an index of the secretion reflex. Patterns of electroencephalograms were studied n comparison with the parotid activity.

1. When the conditioning advanced, the parotid showed two successive activities upon the conditioned stimulus alone. The preceding and succeeding responses were called initial and delayed responses respectively.

2. The delayed response could be regarded as a conditioned reflex, because its latency was almost the same as the time interval between the conditioned and the unconditioned stimulus. The initial response which appeared soon after the onset of the conditioned stimulus could be considered a conditioned reflex, because in our experiment the possibility was completely excluded that the said response might be an unconditioned response to the conditioned stimulus.

3. The electroencephalography could reveal that the delayed response of the parotid was accompanied with a conditioned cortical excitation while the initial response could not be related to a marked changes in EEG at least in the well-conditioned state.

4. In the experiment of generalization, it was found that the initial response was more easily generalized than the delayed response.

5. After the extinction of the established conditioned reflex had been completed, a procedure of disinhibition was applied. Only the initial response of the parotid was elicited by the conditioned stimulus which was applied following the disinhibiting procedure. The recovery of the abolished delayed response was accomplished after the renewed application of the conditioning procedure.

6. Nature of the initial and delayed responses was discussed upon the basis of the electroencephalographic findings.

Our sincere thanks are due to Prof. K. Motokawa for his valuable suggestions and advices during the course of this work.

References

1) Motokawa, K. & Huzimori, B., Tohoku J. Exp. Med., 1949, **50**, 215.
2) Motokawa, K., Tohoku J. Exp. Med., 1949, **50**, 225.
3) Switzer, S. A., J. Exp. Psychol., 1934, **17**, 603.
4) Rodnick, E. H., J. Exp. Psychol., 1937, **20**, 409.
5) Rodnick, E. H., J. Exp. Psychol., 1937, **20**, 507.
6) Iwama, K., Tohoku J. Exp. Med., 1950, **52**, 53.
7) Iwama, K. & Shinjo, T., Tohoku J. Exp. Med., 1950, **52**, 223.
8) Pavlov, I. P., Conditioned reflexes, an investigation of the physiological activity of the cerebral cortex (Trans. by G. V. Anrep), Oxford Univ. Press, London, 1927.
9) Hilgard, E. R. & Marquis, D. G., Conditioning and learning, New York, 1940.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Genetical Studies on Skin Diseases

VIII. Hair Nevus

By

Masao Miura and **Akibumi Komatsu**
(三浦 正男) (小松 昭文)

(From the Department of Dermatology, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. M. Ito)

(Received for publication, May 16, 1952)

In 1924, hair nevus was first described by Fessler[1] under the name of congenital hair tumor as one kind of the teratomas and the second was reported by Nomura[2] in authors' clinic (1938). Since that time, the reports of similar cases in Japan were presented by Yamaguchi-Kuroda and Momose[3] (5 cases). The authors experienced recently two cases of hair nevus and studied especially on the condition of the nerves in the tumor with Bielschowsky-Seto's silver impregnation method.

Report of Cases

Case 1. A 16 years old boy. No similar tumor was found in his families. Examination: There were 3 scallionlike soyabean-sized hard tumor on the frontal region of both tragi (two in the right and one in the left side) and their surface was covered by normal skin.

Case 2. A 9 months old male. His mother stated that his paternal grandmother had a similar tumor in the front of her left tragus which was removed in her childhood and that paternal grandmother's mother had also presented soyabean-sized dark tumor on her right upper eyelid. Examination: There was a scallionlike peasized hard tumor on the frontal region of the left tragus and it was covered by normal skin with rosy tone.

Histological findings. The tumors in both cases are solid and decked by the normal epidermis. Extending from subpapillar layer to subcutis numerous hair follicles are found in various forms from ebryo bud to fully developed one. Most of them, which situate in stratum reticulare, present variously sized cross sections but regular direction of the length axes. Some of them near the epidermis show the longitudianl section and a few of them reach to the skin surface showing normal downy hair. Other some hair follicles are accompanied by the sebaceous gland. In the other hair roots, several holes are found. These holes are equal to what Fessler described under the name of " Gemeinsame äussere Wurzel-

scheide mehrerer Haare" (Momose, 1949). But all of hair roots have never the arrector pili muscles and no sweat glands are found anywhere. In the second case, the subcutaneous fat tissue is comparatively well developed and the netlike connective tissue penetrates into it. In our first case, the cartilage is found in the deeper part of the tumor. Both cases present no figure of the invation of the epidermis as reported by Fessler.

Examination by Bielschowsky-Seto's silver impregnation method. The authors observed the relation between the tumor and the nerves with the Bielschowsky-Seto's silver impregnation method. The nerves are found around the blood vesseles or the hair follicles. Many sensible and fein vegetative nerve bundles run parallelly with the course of blood vesseles. The nerve bundles along the blood vesseles ramify for the hair follicles on this way. The ramified nerve bundles reach to the lower part or the sides of the hair follicles and advance upwards or down around the hair root. Viz. these nerve fibrers run in all directions and sometimes surround semicircularly the hair root. In some cases the nerve fibers look as if they surround circularly all round of the hair root from both sides. Many nerve fibers are generally found in the region between the hair follicle and the sebaceous gland. Even when the relation between the nerves around the hair roots and the blood vesseles cannot be found so clearly, the blood vesseles can more or less be found around the hair follicles and many nerve bundeles run along them. And then the nerve bundles around the hair root present the findings as follows. In some cases, a ramified nerve bundle reaches to the centre of the hair root. In other cases, some nerve fibers run to the hair root parallelly each other or enter radially from all round of the hair root.

These nerve bundles show no abnormal findings as hypertrophy or fissure and a certain relation between the degree of growth of each hair follicle and the number of nerve bundles around it can't be recognized.

Comment

Hair nevus has been already discussed by Fessler, Nomura, Yamaguchi-Kuroda and Momose.

The existence of the cartilage in the tumor was already reported by both Fessler and Momose. The similar finding was found also in our first case. The sweat glands which had been found in the cases by Fessler and Nomura were not present in the cases by Momose and us, while the sebaceous glands were seen more or less in all reports. Fessler and Nomura found the slightly developed subcutaneous fat tissue, but Momose did not recognize them. In our second case, the comparatively well developed subcutaneous fat tissue could be found.

The authors could find no reports in the literature in which the relation between the tumor tissue and the nerves was observed. Seto[4] stated the distributional cource of the nerves in the head skin as follows. "The nerve bundles in the head skin start from the thick bundles in the aponeurosis as the mixed nerves and run upward in the interlobular connective tissue of the subcutis. First of all, they make the primary nervous plexus in the zone where the sweat glands are situated and this plexus combine with the plexus of the vegetative nerves around the blood vesseles. Then they run for the papillary upwards along the arrector pili muscles and make the secondary nervous plexus in the subpapillary. On the way, they give the branches to the hair follicles and these nerve bundles reach to the neuroshell in the follicle neck running horizontally between the arrector pili muscle and the basis of the sebaceous gland, and there ramify to fein fibers. In this part the hair nerve fibers make the end aparatus. On the other hand, the vegetative nerve bundles are rich around the blood vesseles and run along them. They are also rich around the sebaceous glands, the hair follicles and the arrector pili muscles." In our two cases, the course and the distribution of the nerve could not be observed in detail as Seto. The neuroshell in the neck of the hair follicle also could not be found. But it is guessed that the area, where the nerve bundles around the hair roots could be found, agreed with the neuroshell described by Seto. Many nerve bundles around or along the blood vesseles also could be found. Thus the fact that the nerve bundles are very abundant around the hair follicles was recognized.

Prinz[5] described that the development of hair nevus is based on the abnormal growth of the epidermis as the invation of the epidermis reported by Fessler. But no figure as he described could be recognized in our two cases.

Itô[6] stated as follows: "The inheritance on the supernumerary ears or the cartilaginous nevus was recognized by Siemens, Thomas, Rohrer, Hunt, Brander, Jenkins, Teveli, Miller & Miller[7], Ono, Hara, etc. Then it was able to presume that the hair nevus also is inheritable, although there had had no faborable report." In second case the grandmother was removed from the scallionlike soyabean-sized tumor in her childhood, and it gives some sugestion to Itô's presumption.

Summary

Two cases of hair nevus were reported. The authors studied them chiefly with Bielschowsky-Seto's silver impregnation method. The fact that the hair follicles were usually accompanied by the nerve bundles was clearly recognized.

Furthermore in our second case, the grandmother had a similar

tumor in the front of her left tragus in her childhood.

Thus the authors presumed that hair nevus is not mere invation of epidermis as reported by Fessler but is the inheritable ectodermal teratom participated by the nervous system.

References

1) Fessler, Arch. f. Dermat., 1924, **146**, 411.
2) Nomura, Taisei (Japanese), 1938, **35**, 956.
3) Momose, Jap. J. Dermat., 1949, **59**, 16.
4) Seto, Arb. Anat. Inst. Univ. Sendai, 1942, **25**, 99.
5) Prinz, Arch. f. Dermat., 1951, **193**, 513.
6) Ito, Iden-Hifubyo-Gaku (Genetics of Skin Diseases), Tokyo 1949, 120.
7) Miller & Miller, Arch. of Dermat., 1949, **60**, 601.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Experimental Studies on the Effect of Salvarsan Injection upon Vitamin B_1 in Animals

By

Chikakuni Watanabe

(渡辺 睦邦)

(From the Department of Dermatology, Faculty of Medicine, Nihon University, Tokyo; Director: Prof. O. Miura)

(Received for publication March 1, 1952)

Polyneuritis or beriberi-like paralysis as a type of toxic action of the drug salvarsan has been reported by many scholars. But I haven't encountered any other reports on the relation between the drug salvarsan and vitamin B_1 except the one by Saito[1] who studied clinically the vitamin B_1 in the blood and urine before and after the injection of mapharsen. Based on his studies, however, he concluded that there is no definite relationship between them. Therefore, I attempted to study by animal experiments to clear this point, as below.

Experimental

Methods and Materials

1) Animals used for experiments: Normal white rabbits, from 2 to 3 kg. in body weight, were used after feeding for over one week with 100 g. of carrots and sufficient bean curd lees daily. These experiments were carried out by using the rabbits fasted for 16 hours and with each group consisting of 5 to 8 animals.

In the case when the same rabbits were to be used again, the controls, into which physiologic saline solution was injected, and the animals, into which salvarsan was injected 0.05 g./kg., were employed in the experiment after an elapse of over 1 and 2 weeks respectively. The rabbits injected with 0.1 or 0.2 g./kg. of salvarsan were not used again for the reason that the morphological changes in liver and kidneys by the injection of large doses of salvarsan have been proved by Ullmann[2] *et al.*

2) Salvarsan drugs: Neo-ehramisol (Neoarsenobenzol V.P.J.), containing 18.5% of arsenic, was employed. Moreover, as the toxic dose of neosalvarsan for rabbits was found to be 0.15–0.30 g./kg. according to Schlossberger,[3] an average dose of 0.2 g./kg. is considered to be un-

objectionable. The author then dissolved the toxic dose, one-half of the toxic dose and one-fourth of the toxic dose of salvarsan, namely, 0.2, 0.1 and 0.05 g. of salvarsan per kg. of body weight respectively, in distilled water at the rate of 1 cc./kg. and injected into the auricular vein.

3) Quantitative analysis of vitamin B_1: As the author used the thiochrome method[4)5)] using permutit and proceeded with Fujita's[6)] procedure, the details will not be stated here. However, from the results by Nishio and others,[7)] the 8% Taka-diastase solution was used as the enzymatic solution to hydrolyze co-carboxylase.

Preliminary Experiment

1) Study on the physiological variations of the total amount of vitamin B_1 in the blood. With the blood obtained early in the morning from the five fasted rabbits fed as mentioned above, the total amount of vitamin B_1 in the blood was quantitatively measured once a week for 4 to 5 weeks. The variations in the same rabbit are within $\pm 0.8\%$.

2) Influence of physiological saline solution on the total amount of vitamin B_1 in the blood. With the blood obtained from the auricular vein immediately before and 1, 2, 3, 6, 12, 24 hours after a single injection of 1 cc./kg. of physiological saline solution into the auricular vein, the variations of the total amount of vitamin B_1 in this blood were measured. Variations were hardly observed. Then, five injections at 1 cc./kg. were done every other day and with the blood obtained immediately before the first injection and 2 hours after the third and fifth injections the total amount of vitamin B_1 in the blood was measured. The total amount of vitamin B_1 in this blood also was hardly influenced.

Results

I. The influence of salvarsan on the total amount of vitamin B_1 in the blood

Single injection

1) 0.05 g./kg. of salvarsan: The variations of the total amount of vitamin B_1 in the blood before and after the injection are only within $+0.4 \sim -0.7\gamma\%$.

2) 0.1 g./kg. of salvarsan: As shown in Table I, a remarkable decrease is observed 1 and 2 hours after the injection, then the amount gradually returned to normal to reach the previous level after 24 hours.

3) 0.2 g./kg. of salvarsan: As shown in Table I, the amount reached the minimum after 2 hours.

Repeated injections

In the rabbits injected with 0.05 g./kg. of salvarsan five times every

Table I

Influence of Salvarsan on the Total Amount of Vit. B_1 in the Blood

After inj. (hrs.)	Rabbits			Total amt. of vit. B_1 in the blood		Comparison of amounts before and after inj.	
	No.	Weight (g.)	Sex	Before inj. (γ%)	After inj. (γ%)	Variation (γ%)	%
1) Single injection of 0.1 g./kg. of salvarsan							
1	36	2850	m	15.1	12.3	−2.8	81.5
	37	2600	m	13.3	11.6	−1.7	87.2
	24	2650	f	12.5	10.6	−1.9	84.8
	20	2500	m	11.4	9.2	−2.2	80.7
	9	2800	m	9.0	7.8	−1.2	86.7
	Average			12.26	10.30	−1.96	84.01
2	17	2250	f	16.1	13.7	−2.4	85.1
	38	2800	m	11.8	9.6	−2.2	81.4
	39	2150	f	11.7	9.4	−2.3	80.3
	25	2400	m	10.4	8.6	−1.8	82.7
	30	2500	m	9.9	7.6	−2.3	76.8
	Average			11.98	9.78	−2.20	81.64
3	23	2150	f	14.4	13.1	−1.3	91.0
	3	2150	f	13.1	11.2	−1.9	85.5
	40	2650	f	12.9	12.0	−0.9	93.0
	4	2650	f	11.0	10.1	−0.9	91.8
	41	2800	m	9.3	8.5	−0.8	91.4
	Average			12.14	10.98	−1.16	90.44
6	16	2250	m	16.7	16.0	−0.7	95.8
	42	2500	f	15.2	14.0	−1.2	92.1
	43	2600	m	12.8	12.3	−0.5	96.1
	15	2500	m	10.3	9.4	−0.9	91.3
	44	2800	f	9.9	9.0	−0.9	90.9
	Average			12.98	12.14	−0.84	93.53
12	45	2600	m	13.3	13.0	−0.3	97.7
	46	2650	f	12.3	12.4	+0.1	100.8
	2	2800	m	12.0	11.5	−0.5	95.8
	28	2150	f	11.7	10.7	−1.0	91.5
	47	2800	m	9.2	9.1	−0.1	98.9
	Average			11.70	11.34	−0.36	96.92
24	6	2100	m	17.1	17.4	+0.3	101.8
	48	2250	f	16.5	16.0	−0.5	97.0
	13	2800	m	11.6	11.8	+0.2	101.7
	49	2400	m	10.1	10.0	−0.1	99.0
	5	2400	m	9.4	9.0	−0.4	95.7
	Average			12.94	12.84	−0.10	99.23
2) Single injection of 0.2 g./kg. of salvarsan							
2	27	2150	m	13.5	11.0	−2.5	81.5
	50	2250	m	10.8	9.7	−1.1	89.8
	51	2300	m	13.6	11.6	−2.0	85.3
	52	2900	f	15.2	12.5	−2.7	82.2
	53	2700	f	9.4	8.1	−1.3	86.2
	19	2550	m	13.3	10.2	−3.1	76.7
	54	2300	m	11.6	10.0	−1.6	86.2
	12	2100	m	10.7	7.3	−3.4	68.2
	Average			12.26	10.05	−2.21	81.96
6	32	2450	m	17.0	15.5	−1.5	91.2
	55	2350	m	15.3	14.2	−1.1	92.8
	33	2400	m	12.4	12.0	−0.4	96.8
	56	2750	m	11.6	10.3	−1.3	88.8
	57	2050	f	9.2	8.2	−1.0	89.1
	Average			13.10	12.04	−1.06	91.91
12	58	2100	m	16.3	15.1	−1.2	92.6
	29	2550	f	13.2	12.0	−1.2	90.9
	22	2550	m	12.1	11.5	−0.6	95.0
	59	2400	m	10.9	9.7	−1.2	89.0
	35	2350	f	9.8	10.0	+0.2	102.0
	Average			12.46	11.66	−0.80	93.58

other day, the amount was measured immediately before the first injection and 2 hours after the third and fifth injections. As shown in Table II, hardly any variation is observed after the third injection, but a decrease is undoubtedly observed after the fifth injection.

TABLE II

Influence of Salvarsan on the Total Amount of Vit. B_1 in the Blood, when 0.05 g. was Injected Repeatedly per kg. Bodyweight

Rabbits			Total amt. of vit. B_1 before inj. (γ%)	2 hrs. after 3rd inj.			2 hrs. after 5th inj.		
				Total amt. of vit. B_1 (γ%)	Comparison with amt. before inj.		Total amt. of vit. B_1 (γ%)	Comparison with amt. before inj.	
No.	Weight (g.)	Sex			Variation (γ%)	%		Variation (γ%)	%
60	2500	m	19.2	20.0	+0.8	104.2	17.5	−1.7	91.1
1	2650	m	15.1	14.2	−0.9	94.0	13.6	−1.5	90.0
61	2350	m	13.3	13.1	−0.2	98.5	10.9	−2.4	82.0
62	2100	f	11.3	10.7	−0.6	94.7	10.8	−0.5	95.6
63	2250	f	7.4	8.1	+0.7	109.5	5.6	−1.8	75.7
Average			13.26	13.22	−0.04	99.70	11.68	−1.58	88.08

II. The influence of salvarsan on the total amount of vitamin B_1 in the organs

Single injection

The total amount of vitamin B_1 in liver, kidneys, heart, spleen, adrenals, muscle (M. pectoralis major), cerebrum, spinal cord and peripheral nerves (N. ischiadicus, N. tibialis and N. peronaeus communis) of rabbits forced to succumb by artificial hemorrhage 2, 6 and 12 hours after the injection of 0.2 g./kg. of salvarsan was quantitatively measured. As control, the amount in the rabbits forced to succumb by artificial hemorrhage 2 and 12 hours after the injection of physiological saline solution was measured, but no great difference was observed, and therefore they were put into one group. In other parts the amount in each organ decreased 2 hours after the injection of salvarsan and that in the liver and spleen decreased more than 10% after 12 hours compared with the control, but in the muscle and peripheral nerves it returned almost to normal while in the another organs it was situated in between (Table III).

Repeated injections

In rabbits administered with 0.05 g./kg. of salvarsan intravenously five times every other day and then forced to succumb by artificial hemor-

rhage 2 hours after the fifth injection, the amount in the same organs mentioned above was measured. Physiological saline solution was administered in the controls. As shown in Table III, the amount decreased remarkably in the liver and spleen, but in the muscle and peripheral nerves hardly any variation was observed, and in the other organs it was situated in between.

III. Study on the mechanism of vitamin B_1 decrease

As mentioned above, in the rabbits administered with large amounts of salvarsan, the amount of detectable vitamin B_1 in the blood and organs by the thiochrome method decreases. Therefore, in order to clarify this mechanism the following experiments were performed.

1) Study on the direct interaction of salvarsan and vitamin B_1: The amount of vitamin B_1 was measured immediately after adding 1 cc. of 0.2% salvarsan solution to 1 cc. of 1 γ/cc. vitamin B_1 hydrochloride solution. As the average value of the 3 experiments was 0.993 γ, this fact means that salvarsan doesn't change vitamin B_1 directly.

2) Study on the influence of salvarsan on phosphatase: As is widely known, the combined form of vitamin B_1 is not detected by the thiochrome method unless it is turned into a free form by phosphatase. Therefore, if salvarsan can inhibit the action of this enzyme, the measurable total amount of vitamin B_1 in the blood and organs may show a value lower than the actual one. In order to prove this, the following experiments were performed.

a) As mentioned above, the enzymic solution shows the best result at the concentration of 8%. Therefore, the total amount of vitamin B_1 in the blood obtained from the rabbits 2 hours after the administration of 0.2 g./kg. of salvarsan, and to which is added enzymic solution of various concentrations, namely 8, 10, 12, 15 and 20% was measured. But the amount doesn't increase with the increase of the concentration of the enzymic solution.

b) 10 cc. of human blood was divided into two portions of equal volume, and to one of them 1 cc. of 0.2% salvarsan was added and to another 1 cc. of water, then the total amount of vitamin B_1 was measured. Each experiment was repeated three times. The average amount showed a decrease of 8.5% in the former. Therefore, the detectable amount of vitamin B_1 in the blood by the thiochrome method was decreased by salvarsan in vitro also.

c) 2 cc. of 1γ/cc. vitamin B_1 hydrochloride was added to 10 cc. of human serum heated up to 80°C for 15 minutes to inhibit the action of phosphatase, then the solution was divided into two parts. To one was added 1 cc. of 0.2% salvarsan solution and to the other 1 cc. of water.

Table III

Influence of Salvarsan on the Total Amount of Vit. B_1 in Organs

After inj. (hrs)	Rabbits No.	Weight (g.)	Sex	Total amount of vit. B_1 in organs: Liver (γ%)	Kidneys (γ%)	Heart (γ%)	Spleen (γ%)	Adrenals (γ%)	Muscles (γ%)	Cerebrum (γ%)	Spinal cord (γ%)	Pertpheral nerves (γ%)
1) Single injection of 0.2g./kg. of salvarsan												
2	27	2150	m	246	200	276	133	173	34.4	241	193	18.2
	50	2250	m	313	276	299	164	161	37.1	168	203	20.5
	51	2300	m	325	181	345	187	134	41.4	246	241	21.4
	52	2900	f	344	262	270	182	175	53.9	286	178	31.1
	53	2700	f	257	245	301	153	157	43.1	193	174	15.3
	19	2550	m	312	242	336	156	190	49.7	242	212	29.4
	54	2300	m	282	230	285	128	174	45.1	227	201	16.6
	12	2100	m	276	217	266	135	199	47.2	280	188	15.1
	Average			296.6	231.6	297.3	154.8	170.4	43.99	235.4	198.8	20.95
	(%)			(73.8)	(87.9)	(87.6)	(86.7)	(71.2)	(72.7)	(75.5)	(75.6)	(88.9)
6	32	2450	m	368	300	307	172	241	54.5	312	275	30.1
	55	2350	m	310	265	299	174	213	62.3	281	251	21.7
	33	2400	m	323	226	285	146	193	49.7	263	239	21.5
	56	2750	m	285	209	277	139	206	42.8	259	220	20.0
	57	2050	f	273	189	281	152	239	51.1	261	214	17.0
	Average			311.8	237.8	289.8	156.6	218.4	52.08	275.2	239.8	22.06
	(%)			(77.6)	(90.3)	(85.4)	(87.7)	(89.4)	(86.1)	(88.3)	(91.2)	(93.5)
12	58	2100	m	362	310	355	162	274	71.3	335	271	29.8
	29	2550	f	407	271	338	172	250	65.7	318	232	20.5
	22	2550	m	329	241	320	149	216	52.0	289	251	31.0
	59	2400	m	337	216	344	152	205	51.1	256	248	17.6
	35	2350	f	274	242	275	153	221	57.2	269	247	18.6
	Average			341.8	256.0	326.4	157.6	233.2	59.46	293.4	249.8	23.50
	(%)			(85.1)	(97.2)	(96.2)	(88.2)	(95.5)	(98.3)	(94.1)	(95.0)	(99.6)
2) Control of 1) (Physiological saline was substituted for salvarsan)												
2	64	2500	f	456	213	387	—	275	71.6	345	—	—
	65	2500	m	391	330	338	182	204	45.1	274	281	31.5
	66	2150	f	350	249	326	165	230	58.4	294	250	16.5
	67	2450	m	382	239	353	152	252	61.0	315	223	24.7
	68	2000	m	433	275	286	197	284	76.7	337	299	22.0
	Average			402.4	261.2	338.0	174.0	249.0	62.56	313.0	263.3	23.68
12	69	2150	m	466	305	399	192	279	62.6	338	280	28.7
	70	2300	m	442	281	344	193	261	69.5	311	254	24.6
	71	2100	m	409	266	307	187	228	47.0	298	252	21.1
	72	2650	f	361	253	328	156	210	55.1	288	239	20.9
	73	2500	m	327	223	235	183	219	57.9	317	289	22.3
	Average			401.0	265.6	340.6	182.2	239.4	58.42	310.4	262.8	23.52
Total average				401.7	263.4	339.3	178.6	244.2	60.49	311.7	263.0	23.59

3) Repeated injections of 0.05 g./kg. of salvarsan

60	2500	m	355	358	361	172	266	57.1	325	273	25.9
1	2650	m	312	309	313	167	308	58.4	331	267	20.6
61	2350	m	327	231	296	145	231	66.7	292	206	26.2
62	2100	f	341	207	215	154	203	46.8	304	208	24.4
63	2250	f	301	200	247	112	219	41.6	246	171	14.7
Average			327.2	261.0	286.4	150.0	245.4	54.12	299.6	225.0	22.36
(%)			(82.2)	(92.9)	(89.6)	(86.7)	(90.7)	(91.2)	(89.5)	(88.6)	(96.8)

4) Control of 3) (Physiological saline was substituted for salvarsan)

74	2550	f	448	390	380	196	402	69.3	400	311	31.4
75	2400	m	392	241	345	180	215	68.7	296	254	31.1
76	2500	m	409	354	347	152	205	44.6	299	267	22.3
77	2350	f	380	208	302	183	300	71.1	358	209	14.8
78	2300	f	347	211	224	154	231	42.9	321	229	15.9
Average			395.2	280.8	319.6	173.0	270.6	59.32	334.8	254.0	23.10

TABLE IV

Total, Free and Combined Forms of Vit. B_1 in Liver

	No.	Total (γ%)	Free Form (γ%)	Combined form (γ%)
After salvarsan injection	1	400	81	319
	2	338	65	273
	3	326	70	256
	4	259	68	191
	5	251	49	202
	Average	314.8	66.6	248.2
Controls	6	456	78	378
	7	431	69	362
	8	378	68	310
	9	374	62	322
	10	368	64	304
	Average	401.4	68.2	335.2

The amount of vitamin B_1 in both solutions was measured without adding the phosphatase solution. By the average of 3 experiments, the amount in the former is 0.843γ, but in the latter 0.993γ. Therefore, the salvarsan added solution decreases about 15%.

3) The amount of urinary excretion of vitamin B_1: The amount of vitamin B_1 in the urine obtained 2 hours before and 2 hours after the intravenous administration of 0.2 g./kg. of salvarsan by catheterisation was measured. By the average of 5 experiments, the former shows 6.820γ, the latter 6.668γ, the average in the latter being decreased slightly and varying with each case. A definite influence of the administration of

salvarsan is not observed.

4) Combined form and free form of vitamin B_1 in the liver: The amount of vitamin B_1 was measured in the liver of rabbits succumbed by artificial hemorrhage 2 hours after the intravenous administration of 0.2 g./kg. of salvarsan. The amount of the free vitamin B_1 hardly varies, the combined form only decreasing, showed in Table IV, as the difference between total and free vitamin B_1 is regarded as the combined form of it.

5) Influence of the addition of cystine and cysteine: 1 cc. of a 20 mg% cystine or a 20 mg.% cysteine solution was added to 5 cc. of 1:20 dilute saline extracts of a rabbit liver excised after 2 hours from the administration of 0.2 g./kg. of salvarsan and after standing the mixtures at room temperature for 30 minutes to 1 hour, the total amount of vitamin B_1 in them was measured. There was no influence seen at all in the average analyses of three experiments.

Summary and Discussion

Summarizing the above, the amount of vitamin B_1 in the blood hardly changes by the administration of 0.05 g./kg. of salvarsan, but when 0.1 g /kg. was administered it decreases to the minimum 1 to 2 hours after and then it gradually returns to normal and reaches the previous state after 24 hours. If 0.2 g./kg. was applied, the amount of it in the blood decreases to the minimum in the same ratio as in the former and the sign of the recovery appears after 6 to 12 hours. In the organs, too, it decreases to the minimum 2 hours after, and in the cases of liver and of spleen the recovery of it is not marked yet after 12 hours, in the other organs, on the contrary, the amount of it returns almost to the previous state at that time.

In reviewing the literature on excretion and deposition of salvarsan, Fischer and Hoppe,[8] to begin with, Fränkel-Heiden and Navassart,[9] Ullman,[10] Riebes,[11] Del Baere,[12] Hashimoto[13] and Seki[14], *et al.*, have proven that salvarsan is excreted very rapidly. And according to Bornstein,[15] Ullmann,[16] Stühmer,[17] Jancso,[18] Murayama,[19] Inosaka,[20] Ōki,[21] *et al.*, the deposition of large amounts of salvarsan in the reticulo-endothelial cells and liver cells for a long time was proved. The results of my experiments also showed that the total amount of vitamin B_1 does not change in case of repeated injections of 0 05 g./kg. every other day until the third injection, and after the fifth injection it decreases only in the organs rich in reticulo-endothelial cells such as liver and spleen. Therefore, it seems that the amount of vitamin B_1 in the body decreases in the presence of large amounts of salvarsan or in the tissues containing large amount of salvarsan.

Then, by what mechanism does such a decrease of vitamin B_1 take place? Indeed, the total amount of vitamin B_1 does not increase by adding large amounts of phosphatase to the blood of the rabbits administered with 0.2 g./kg. of salvarsan and although in vitro total amount of vitamin B_1 in the blood decreases to 91.5% by adding salvarsan to the blood, but the amount of free form of vitamin B_1 also decreases when the salvarsan is added to the serum in which the activity of phosphatase is inhibited and then vitamin B_1 hydrochloride is mixed previously.

Based on the three reasons mentioned below, it may be denied that salvarsan alters vitamin B_1 directly through its physical or chemical action. Firstly, the amount of vitamin B_1 does not change at all after several minutes following the addition of salvarsan direct to the vitamin B_1 solution; secondly, it decreases only up to 85% even by the addition of 2 mg. of salvarsan to 1γ of vitamin B_1 in the experiments on serum in vitro; and thirdly, the decreasing ratio of the total amount of vitamin B_1 in the blood of the rabbits administered with 0.1 g./kg. and 0.2 g./kg. of salvarsan nearly equal each other.

When averaged the figures in the five experiments indicate that the urinary excretion of vitamin B_1 after the administration of salvarsan decreases slightly. But this amount is very little and sometimes a reversed result is shown. Therefore, it may be denied that the decrease of vitamin B_1 in the body depends upon the urinary excretion.

Measuring the amount of total and free form of vitamin B_1 in the liver, the amount of total vitamin B_1 decreases in case of administration of salvarsan, but the amount of the free form is hardly different from the control. Therefore, it is concluded that the decrease of vitamin B_1 depends on the combined form. This coincides with Shimazono's[22] opinion that the amount of the free form of vitamin B_1 hardly varies in the body.

Takaoka,[23] Minami and Takaoka[24] reported that glutathione, one of the redox system in the body as well as cystine and cysteine, decreased by the administration of salvarsan. On the other hand, Hamamoto,[25] Ida,[26] *et al.* reported that the vitamin B_1 turns to negative by the thiochrome method in the cerebrospinal fluid but recovers to a certain degree by the administration of cysteine. Therefore, it may be conceivable that if cystine and cysteine decrease by the administration of salvarsan so that vitamin B_1 turns to negative by the thiochrome method, at least one part of it can recover. No change was observed in the liver extract of rabbits to which was added separately cystine and cysteine after the injection of salvarsan.

Fischer and Hoppe,[3] Bornstein,[15] Engwer,[27] *et al.* reported that salvarsan is excreted in the intestines, too, while on the other hand, Kimura, Hayashi, Chachin and Tanaka[28][29][30] proved the presence of bacilli which can synthesize vitamin B_1 in the intestines. Therefore, salvarsan

excreted in the intestines may attack these bacilli and inhibit the synthesis of vitamin B_1, so that it should be considered that the amount of vitamin B_1 decreases for this reason. But this can be answered sufficiently by Saito's[1] experiment that the amount of vitamin B_1 synthesized by 1 g. of bacilli is only 1γ.

From the reasons mentioned above, the amount of vitamin B_1 in the body, undoubtedly, decreases by the administration of large amounts of salvarsan, or by repeated administrations of relatively small amounts.

I intended to make clear this mechanism and reached the hypothesis that this mechanism may not be due to the direct action of salvarsan on vitamin B_1 but due to another kind of mechanism, or in other words due to the indirect action through the body constituents.

Based on the above animal experiments, it might not be denied that one of the toxic effects of salvarsan depends upon the decrease of vitamin B_1. Therefore, it may be concluded that the administration of vitamin B_1 has a great significance in the prevention and the therapy of the toxic effects of salvarsan.

References

1) Saito, Acta dermat. (Jap.), 1950, **46**, 313.
2) Ullmann, Wien. Kl. Wschr., 1914, **27**, 838.
3) Schlossberger, Kolle-Zieler's Handbuch d. Salvarsantherapie, I, Urban & Schwarzenberg Berlin & Wien, 1924.
4) Hennessy & Cerecedo, Journ. Amer. Chem. Soc., 1939, **61**, 179.
5) Hennessy, Ind. & Eng. Chem., 1941, **14**, 216.
6) Fujita, Jap. Journ. of Med. Prog. (Jap.), 1948, **35**, 473.
7) Nishio, Fujiwara & Kitamura, Vitamin (Jap.), 1948, 1949, **1**, 232, 288.
8) Fischer & Hoppe, Münch. med. Wschr., 1910, **57**, 1530.
9) Fränkel-Heiden & Navassart, Berlin. Kl. Wschr., 1911, **48** (II Halbjahr), 1367.
10) Ullmann, Arch. f. Dermat., 1911, **114**, 511.
11) Riebes, ibid., 1913, **118**, 757.
12) Del Baere, Journ. Exp. Med., 1926, **48**, 24.
13) Hashimoto, Osaka Iji Ss. (Orig.) (Jap.), 1935, **6**, 323.
14) Seki, Journ. of the Jap. Obst. & Gynecol. Soc., 1951, **3**, 74.
15) Bornstein, Dtsch. med. Wschr., 1911, **37**, 112.
16) Ullmann, Wien. Kl. Wschr., 1913, **26**, 929, 978.
17) Stühmer, Arch. f. Dermat. 1914, **120**, 589.
18) v. Jančso, Journ. Exp. Med., 1928, **61**, 63.
19) Murayama, Hukuoka acta med. (Jap.), 1932, **25**, 1137.
20) Inosaka, Jūzenkai Z. (Jap.), 1938, **43**, 1589, 1597, 1615.
21) Ōki, Nagoya Igk. Z. (Jap.), 1940, **51**, 257.
22) Shimazono, Vit. B group & Vit. B_1 (Jap.), Nihon Igaku Shoseki Co. Tokyo, 1950.
23) Takaoka, Dermat. & Urol. (Jap.), 1941, **9**, 493.
24) Minami & Takaoka, Jap. Journ. of Dermat. & Venereol. (Jap.), 1943, **53**, 231.
25) Hamamoto, Vitamin (Jap.), 1948, **1**, 157.
26) Ida, ibid., 1950, **2**, 180.

27) Engwer, Münch. med. Wschr., 1917, **64**, 446.
28) Kimura, Chachin & Tanaka, Vitamin (Jap.), 1948, **1**, 152.
29) Kimura & Hayashi, ibid., 1948, **1**, 271, ibid., 1949, **2**, 82, 88.
30) Hayashi, ibid., 1949, **2**, 40.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Bacteriological Study of Suture Layers in Gastric Cancer and Ulcer

By

Yoshimasa Ushigome

(牛込 善正)

(From Prof. M. Muto's Surgical Clinic, Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, May 20, 1952)

Although bacteriological studies of gastric cancers and peptic ulcers during operation have been made by Meyeringh[1] and others,[2-7] only Friedrich and Weber[4] have reported as to the bacteriology of each suture layers of gastroenterostomy. Because of their neglecting quantitative estimation of microorganisms, I concentrated my efforts on the qualitiative and quantitative bacteriological estimation of gastric and intestinal contents, in each suture layers of anastomosis and the resected stumps, occurring in 108 gastric operations (78-gastric cancers and 30-peptic ulcers).

Methods: The location for examination was swabbed with a piece of sterile cotton fixed at one end of a rod, and the swab was cultured on 3% agar plates, 3% human blood agar plates and 0.5% glucose broths; the media were kept at 6.8 to 7.0 pH and the growth was examined 24 or 48 hours after incubation. If cultures proved negative on agar plates, but positive in broths, further plate cultures were made with the broth culture to determine the type of microorganisms. The bacteriological identification depended upon the type of colony on plate cultures and the characteristics of methylene blue and Gram stainings.

All anaerobes were first cultured in Zeissler's[8] cooked liver broth for about one week, then heated at 80°C for 20 minutes, and transplanted to Zeissler's human blood glucose plates. Anaerobiosis was obtained in a tightly sealed jar, from which the air was removed by means of a vacuum pump. The cultures were maintained at 38°C for 48 hours to isolate microorganisms.

Cases in which no organisms were found to develop on any of the above mentioned culture media will be referred to as "sterile," while cases in which microorganisms were found either to have formed colonies on plate cultures ("plate-positive") or to have developed on no plate but in broth cultures ("broth-positive") will be referred to as "positive." The following terms represent the abundancy of organisms: "scanty"

for the number of colonies less than 10, " moderate " for 11 to 50, " fairly " abundant for 51 to 100, and " abundant " for more than 100.

Results of Examinations of the Gastric and Intestinal Contents and the Suture Layers of the Anastomoses

Of the 108 gastroenterostomies bacteriologically examined, 66 were for gastric cancers (52 gastric resections, 14 non-resections), and 30 were for peptic ulcers, resected in all cases. In most cases, resection was performed after Billroth II and then gastrojejunostomia antecolica oralis inferior with the following three suture layers: continuous mucous suture of catgut (" first suture layer "), seromuscular suture of silkworm-gut (" second suture layer ") and Lembert's serous suture (" third suture layer "). In the cases of simple gastrojejunostomy the three suture layer method was adopted.

The results of bacteriological examinations of the gastric and intestinal contents and of the suture layers may be divided in three groups: 1) 66 gastric cancer, 2) 30 peptic ulcer and 3) 12 gastric cancer cases with preoperative hydrochloric acid treatment. Among the number of positive

TABLE I

Microorganisms Cultured from Various Locations of Gastroenterostomy

Lesion	Location examined	No. of cases examined	Positive culture No. of case (%)	B. coli	B. lacticum	Strept. viridans	B. welchii	Strept. hemolyticus	Saccharomyces	Gram-positive diplococci	Strept. nonhemolyticus	Staph. albus	B. subtilis	B. alcaligenes faecalis	Gram-negative diplococci	Staph. aureus	B. pyocianeus
Gastric cancer	Gastric contents	66	65 (98)	55	17	16	14	9	9	8	6	5	5	4	3	0	1
	Intest. contents	66	66 (100)	59	11	9	11	6	4	5	5	7	3	6	2	1	1
	I. suture layer	66	65 (98)	58	10	11	2	8	4	5	4	3	2	4	2	1	1
	II. suture layer	60	54 (90)	45	5	7	0	5	1	2	2	1	0	4	1	0	0
	III. suture layer	66	54 (52)	29	5	3	0	1	0	1	1	0	2	1	0	0	1
Peptic ulcer	Gastric contents	30	18 (60)	7	1	1	2	1	1	3	1	2	1	0	2	1	0
	Intest. contents	30	18 (60)	12	1	1	3	1	1	1	1	2	1	0	2	1	0
	I. suture layer	30	14 (47)	4	1	1	0	1	1	1	1	3	1	0	2	1	0
	II. suture layer	29	7 (24)	3	0	1	0	0	0	0	1	1	0	0	0	1	0
	III. suture layer	30	4 (13)	1	0	0	0	0	1	0	0	1	1	0	0	1	0
Gastric cancer, HCl-treated	Unreated gastric juice	8	8 (100)	7	4	1	0	3	1	0	0	0	0	1	1	1	0
	Gastric contents	12	10 (83)	5	4	1	2	4	0	0	0	0	0	1	1	1	0
	Intest. contents	12	12 (100)	5	5	1	0	3	0	0	0	0	0	1	1	1	0
	I. suture layer	12	10 (83)	3	3	1	0	3	0	0	0	0	0	1	0	1	0
	II. suture layer	7	6 (86)	1	1	1	0	2	0	0	0	0	0	0	0	0	0
	III. suture layer	12	2 (17)	0	1	0	0	1	0	0	0	0	0	0	0	1	0

cultures obtained and the kind of microorganisms detected, presumably pathogenic ones are Strept. hemolyticus, B. welchii, Staph. aureus and B. coli (Table I). B. coli predominated in the stomach and intestine, B. lacticum was not infrequently found in cancer cases, and some difference was seen in percentage of positive cultures between cancer and ulcer cases.

The results of quantitative estimation of organisms are listed in Table II, where figures with regard to B. coli are given in parenthesis.

Gastric cancer cases. The gastric contents yielded positive plate cultures in 98%, and from the intestinal contents in 100% (Table I), representing more colonies than the gastric contents (Table II). The combination of microorganisms detected from single cultures was: from the gastric contents, 1 kind in 12, 2 in 25, 3 in 21, and 4 in 7 cases; from the intestinal contents, 1 kind in 23, 2 in 27, 3 in 14, and 4 in 2 cases. When more than two kinds of organisms were present in a culture, B. coli was almost always found (Table I).

As for the anastomosis, the percentage of positive cultures obtained from the first suture layer was about the same as those from the gastric and intestinal contents, while microflora were smaller in quantity. The combination of organisms in single cultures was: 1 kind in 27, 2 in 28, 3 in 19, and 4 kinds in 1 cases. From the second suture layer positive cultures were obtained in 90% of the cases, the combination of organisms being 1 kind in 36, 2 in 17, and 3 kinds in 1 cases, whereas the third suture layer gave positive cultures only in 34 cases (52%), of which 15 were broth-positive. The number of colonies in plate cultures decreased markedly and the cases in which 2 kinds of organisms existed in single cutltures were only 6, the remaining 28 cases developing B. coli alone.

Peptic ulcer cases. In peptic (gastric and duodenal) ulcers, 60% of the cases gave positive cultures (broth-positive 30% and plate-positive 30%) from the gastric contents. Similar results were obtained with the intestinal contents, 60% of the cases (broth-positive 27% and plate-positive 33%) being positive cultures. The combination of organisms in single cultures were: from the gastric contents, 1 kind in 11, 2 kinds in 6, and 3 kinds in 1 case; from the intestinal contents, 1 kind in 9, 2 in 7, and 3 kinds in 2 cases. *B. coli* was absent from the gastric in 23 cases (77%), and from the intestinal contents in 18 cases (60%).

As for the anastomosis, the rate of positive cultures varied from 47% at the first suture layer to 24 and 13% at the second and third layers respectively. The percentage of positive broth cultures and the number of colonies on plates decreased markedly in the order of the first, second and third suture layers (Table II).

Gastric cancer cases with preoperative hydrochloric acid treatment. In these

TABLE II

Quantitive Observations of Microorganisms Cultured from Various Locations of Gastroenterostomy

The number of B. coli are shown in parentheses.

Lesion	Location examined	No. of cases examined	Number of cases of						
			Cultures			Colonies on plates			
			Sterile	Broth-positive	Plate-positive	Scanty	Moderate	Fairly abundant	Abundant
Gastric cancer	Gastric contents	66	1 (11)	0 (2)	65 (53)	4 (10)	8 (10)	14 (10)	39 (21)
	Intest. contents	66	0 (7)	0 (0)	66 (59)	2 (5)	7 (8)	9 (12)	48 (34)
	I. suture layer	66	1 (8)	7 (10)	58 (48)	11 (16)	11 (5)	12 (18)	24 (9)
	II. suture layer	60	6 (15)	7 (13)	47 (32)	10 (17)	10 (10)	18 (4)	9 (1)
	III. suture layer	66	32 (37)	15 (16)	19 (16)	14 (12)	2 (4)	3 (0)	0 (0)
Peptic ulcer	Gastric contents	30	12 (25)	9 (5)	9 (2)	1 (0)	5 (2)	1 (0)	2 (0)
	Intest. contents	30	12 (18)	8 (7)	10 (5)	1 (2)	3 (2)	2 (1)	4 (0)
	I. suture layer	30	16 (26)	7 (2)	7 (2)	3 (2)	3 (0)	1 (0)	0 (0)
	II. tuture layer	29	22 (26)	3 (2)	4 (1)	2 (1)	2 (0)	0 (0)	0 (0)
	III. suture layer	30	26 (29)	1 (0)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)
Gastric cancer, HCl-treated	Untreated gastric juice	8	0 (1)	0 (0)	8 (7)	0 (1)	0 (1)	2 (0)	6 (5)
	Gastric contents	12	2 (7)	1 (3)	9 (2)	2 (1)	3 (0)	2 (1)	2 (0)
	Intest. contents	12	0 (7)	2 (0)	10 (5)	2 (1)	3 (2)	0 (1)	5 (1)
	I. suture layer	12	2 (9)	2 (0)	8 (3)	2 (2)	5 (1)	1 (0)	0 (0)
	II. suture layer	7	1 (6)	2 (0)	4 (1)	4 (1)	0 (0)	0 (0)	0 (0)
	III. suture layer	12	10 (12)	0 (0)	2 (0)	2 (0)	0 (0)	0 (0)	0 (0)

cases, the gastric lavage was made 3 to 4 hours before operation, followed by the instillation of 150 cc. of 0.2 or 0.4% hydrochloric acid solution. Positive cultures were obtained in 83% of the gastric contents, thus being much lower than in the cancer cases not treated with hydrochloric acid (Table I). The cultures in the acid-treated cases showed a decrease both in number and in kind of microorganisms as compared with those in untreated cases (Table I, II). Bacteriological examination of the gastric juice before acid instillation was also made, though no distinct difference in percentage of positive cultures was seen between the contents of the acid-treated stomach and the gastric juice before treatment, organisms detected were less in number in the former than in the latter.

From the intestinal contents, all the cultures yielded positive results, but the number and kind of organisms were smaller than in the untreated cases.

As for the anastomosis, the three suture layers, especially the third, gave positive cultures in percentages similar to those corresponding in the untreated cancer cases, but quantitatively they represented the results similar to those corresponding in the ulcer cases.

To give discussions on the above and conclusions therefrom:—

a) Gastric and intestinal contents in cancer and ulcer cases. In cancer cases, cultures from gastric and intestinal contents represented a higher percentage of positiveness and a larger number of organisms than ulcer cases (Table I). The percentages of positive cultures reported by authors on the gastric and intestinal contents in gastric cancer and peptic ulcer cases are listed in Table III. My results are in substantial agreement with those of previous authors, especially in that the percentage of positive cultures of both microflora in general; B. coli alone is higher in cancer than in ulcer cases.

TABLE III

Summary of the Results Obtained by Various Investigators of the Percentage of Positive Cultures from Gastric and Intestinal Contents

Location examined	Author	Gastric cancer			Peptic ulcer		
		No. of cases examined	Positive with		No. of cases examined	Positive with	
			Micro-organisms in general %	B. coli %		Micro-organisms in general %	B. coli %
Gastric contents	Meyeringh (1925)	28	100	83	49	53	4
	Bitter & Lohr (1926)	44	81	73	85	54	4
	Tatebayashi (1930)	71	100	97	150	31	7
	Friedrich & Weber (1932)	8	100	65	17	53	0
	Yamaguchi (1933)	300	98	65	70	66	9
	Seley & Colp (1941)	16	94	38	24	46	0
	Nei (1941)	12	92	75	8	63	34
	Ushigome (1949)	66	98	83	30	60	23
Duodenal contents	Bitter & Lohr (1926)	44	99	75	92	51	3
	Yamaguchi (1933)	300	96	65	63	71	19
	Ushigome (1949)	66	100	89	30	60	40

Of the organisms detected, Gram positive and negative diplococci, Staph. albus, Staph. aureus, B. subtilis, etc. were found in cancer case in about the same small percentage as in ulcer cases, while B. coli, B lacticum, Strept. viridans, Strept. hemolyticus, B. welchii, Saccharomyces, etc. were scanty in ulcer cases; B. alcaligenes faecalises absent. These, results are similar to previous reports that a large number and many kinds of organisms were found in cancer cases, whereas in ulcer cases cultures were either sterile or contained only nonpathogenic organisms.

Tatebayashi[3] emphasized that there was little difference in kind of organisms contained in the stomach due to the preoperative acid treatment, while the number of organims, especially of B. coli and its percentage of positiveness, showed a decrease due to the treatment. The same tenden-

cy occurs in my acid-treated cases.

b) Suture layers of the anastomosis. In gastric cancer cases, the percentage of positive cultures from the first suture layer was as high as that of the gastric and intestinal contents. This rate markedly decreased in the order of the first, second and third layers. It will be understood from Table II that the preoperative instillation of hydrochloric acid is effective in diminishing microorganisms.

Making similar bacteriological examinations of the suture layers of the anastomosis (the mode of suture being the same as that adopted in our clinic), Friedrich and Weber found that the first, second and third suture layers yielded positive cultures, in 8 cancer cases, in 100, 88 and 88 (as to B. coli 63, 50 and 38%) respectively, and in 17 ulcer cases in 53, 41 and 0% respectively (B. coli absent from each layer). It may be concluded from these and my results that the third layer is sterile in ulcer cases, while in cancer cases there are not infrequently found microorganisms.

Bacteriological Examination of Gastric and Duodenal Stumps

In 42 cancer and ulcer cases, the stumps, made either with a knife or by electrocautery between two lines of metal clamps set by means of Petz's suture apparatus, were examined bacteriologically. The gastric stump was sterile in 48% of cancer cases (B. coli 64%), and in 83% of ulcer cases (B. coli 93%). In gastric cancers, the 22 cases of positive cultures obtained from the gastric stump consisted of 6 broth-positive and 16 plate-positive ones with scanty colonies, comprising B. coli (15), B. lacticum (4), Strept. viridans (3), Staph. albus (3), and Strept. nonhemolyticus (1 cases). In ulcers, of the 5 positive cases obtained from the gastric stump 4 were broth-positive and 1 was plate-positive with scanty colonies; microorganisms detected were B. coli (2), Staph. albus (1), Gram-positive diplococcus (1), and Saccharomyces (2 cases).

From the duodenal stump in gastric cancer cases, I obtained positive cultures in 50% of the cases (10 broth-positive and 11 plate-positive cases), developing B. coli (12), B. lacticum (5), Strept. viridans (3), Gram-negative diplococcus (4), Staph, albus (2), and Strept. hemolyticus (1 cases), whereas in ulcer cases, positive cultures were obtained in 32% of the cases (3 broth-positive and 4 plate-positive cases), containing B. coli (2), Staph. albus (2), Gram-negative diplococcus (2), and Strept. viridans (1 cases).

It is noted that both in cancer and in ulcer cases the percentage of sterile cultures obtained from both stumps was a little higher when resection was made by electrocautery than with a knife.

Furthermore, when the duodenal stump was buried by the continuous serose suture and Lambert's suture, cultures from the suture layers proved to be sterile in 95% of cancer and 100% of ulcer cases.

The Bacteriological Examination and Its Bearing on the Results of the Operation

Friedrich and Weber state that death following gastric resection for cancers is mainly due to peritonitis, which may develop, depending upon the number and kind of microorganisms contaminating the peritoneum during the operative procedure. Thus, much danger is to be apprehended, according to Yamaguchi,[5] if B. coli, B. welchii and Strept. hemolyticus are present.

As previously stated, a relatively large number and many kinds of organisms are found in the gastroenterostomy for gastric cancer. Although in our operations all precautions were taken to prevent the leaks of gastric and intestinal contents and to minimize the contamination of peritoneum and yet, plate-positive cultures were obtained from the third suture layer in 19 of the 66 cancer cases (Table II). The patients in these cases, however, could recover without complication of peritonitis. This implies that even cancerous patients are able to suppress microflora contaminating the peritoneum to a certain degree. Practically, therefore, there would be little danger of producing peritonitis due to the contamination during the operation, if the operation should be performed carefully.

In the 108 cases examined, 3 cases of complications and 6 fatal cases occurred after the operation for cancers. In the first of the 3 cases, the complication was peritonitis, which was due to the transversostomy following the simultaneous operation of excising the transversal colon, and the patient was cured. In the second cases, the complication was also peritonitis, which was found on post-mortum examination to be due to a perforation of rice grain size formed about 2 cm. below Lembert's suture layer of the front wall of the duodenum; in this case, both the closed duodenal stump and the third layer of suture layer of anastomosis on the operation were found to be sterile. In the third case, the complication was found re-operation to be due to the stenosis of the transversal colon, in which the bacteriological examination on the third layer of anastomosis resulted in sterile. In the other 4 deceased patients, in which death occurred on the day of or in a few days after operation, the deaths might be ascribed to their inability of resisting the operation, for the contamination of the peritoneum by microflora during the operation was slight and peritonitis was denied clinically in these cases.

Summary

1. From gastric and intestinal contents at the gastrojejunostomy B. coli and other organisms are cultured in all 66 cases of gastric cancer, and in 60% of 30 gastric ulcer cases, while the kind and quantity of examined organisms in ulcer was less than in cancer.

2. Although the percentage of positive cultures from the mucous suture was almost the same as that of the gastric and intestinal contents, the positive percentage from the serous suture decreased remarkably both in cancer (positive 52%) and in ulcer (positive 13%).

3. Duodenal stumps after serous suture were steril in all 29 ulcer cases and in 95% of 42 cancer cases. Even in positive cases of cancer the quantity of organisms was very few.

4. The microflora contaminating of peritoneum is relatively high at the gastroenterostomy during gastric resection-procedure, but not so high as to produce peritonitis, when the operation was performed carefully.

References

1) Meyeringh, H., Mitt. a d. Grenzgeb. d. Med. u. Chir., 1925, **38**, 149.
2) Bitter, L. and Lohr, W., Arch. f. K. Chir., 1926, **139**, 64.
3) Tatebayashi, Y., Dtsch. Z. Chir., 1930, **224**, 201.
4) Friedrich, R. and Weber, H., Arch. f. Kl. Chir., 1932, **171**, 673.
5) Yamaguchi, H., Nippon Gekagakkai Zassi, 1933, **35**, 49.
6) Seley, G.P. and Colp, R., Surg. etc, 1941, **10**, 269.
7) Nei, N., Nagasaki Iagku Zassi, 1941, **19**, 1717.
8) Zeissler, J., Handbuch d. mikrobiol. Technik, 2, Berlin, 1923, p. 961.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Bacteriological Study in Stomach- and Intestinal-operation

By

Shinichi Shida

(志田 信一)

(From Prof. M. Muto's Surgical Clinic, Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, May 20, 1952)

Succeeding Ushigome's work,[1][2] I have undertaken bacteriological researches in 88 gastric operations (56 gastric cancers and 32 gastro-duodenal ulcers) and 62 intestinal operations. Though most of the results on gastric operations by Ushigome were obtained by the method of Billroth II, herein are presented the results of gastric operations, comparing Billroth I and Billroth II cases to supplement Ushigome's report, and the intestinal operations, not touched upon by Ushigome.

Methods and Judgment; See Ushigome's report.

I. Results of the Examination of Gastric and Intestinal Contents and the Suture Layers of the Anastomosis in Both Billroth I and II Cases

Gastric cancer cases

56 cases of gastric cancer were divided into two groups; the first consisted of 28 cases having gastric resection by the method of Billroth I (25 gastro-duodenostomies and 3 oesophago-duodenostomies with total gastric resection), and the second of 28 cases having gastric resection by the method of Billroth II (22 gastro-jejunostomies, 1 oesophago-jejunostomy with total gastric resection and 5 simple gastro-enterostomies). In the first group, the two suture layer method was adopted and in the second group, the three suture layer method was adopted as described in Ushigome's report.

In gastric and intestinal contents at the place of anastomosis there was no significant difference in percentage of positive cultures, and kinds and quantity of microorganisms in Billroth II and I cases.

In the contents of jejunum in Billroth II group no microorganisms were detected in 2 cases but some appeared in 26 cases (92.8%), in which 19 cases (67.8%) yielded positive plate-cultures and 7 (25.0%) of positive broth-cultures. B. coli were cultured positively in 17 cases (60.7%) and

13 of them (46.4%) were plate-positive. In about half of the cases with more than two kinds of organisms in a culture, B. coli was always detected.

From duodenal contents of Billroth I group, only 1 case showed no microorganisms and the other 27 (96.4%) had some, in which 20 (71.4%) were plate-positive and 7 (25.0%) were broth-positive. B. coli was cultured positively in 10 cases (35.7%) and 6 (21.5%) of them were plate-positive. In short, the kinds of microorganisms detected in the upper part of jejunum were analogous to that in duodenum, but B. coli in the latter was a little smaller both in the percentage of positive cultures and quantity than in the former.

As for the suture layer of anastomosis, the percentage of positive cultures, and kind and quantity of the organisms detected at the serosa-suture were all reduced in Billroth I and II cases from them at the mucosa-layer. For example from the serosa-suture of Billroth II cases no microorganism was detected in 13, and some organisms were seen in 15 cases (53.6%) and in which 6 (21.5%) were plate-positive and 9 (32.1%) of broth-positive. B. coli was detected in 9 cases (32.1%) and 2 (7.2%) of them were plate-positive. From the serosa in Billroth I cases, 10 had no microorganisms detected and 18 (64.3%) had some, in which 8 (28.6%) yielded positive plate-cultures and 10 (35.0%) positive broth-cultures. B. coli was detected in 6 cases (21.5%) and 2 (7.2%) of them were plate-positive.

Gastroduodenal ulcer cases

Among the 32 cases of gastroduodenal ulcer, 7 were operated by Billroth I, 19 by Billroth II, 4 by Finsterer's method and 2 had oesophagojejunostomy after total gastric resection.

Therefore 25 cases except 7 Billroth I group cases were named as Billroth II group.

As the results of examinations gastric contents at the places of anastomosis in 25 Billroth II group cases, 8 had no microorganisms while 17 (68.0%) had some, and B. coli was cultured positively in 7 cases (28.0%). Among 7 cases of Billroth I group, 1 showed no microorganism and 6 (85.7%) had some, and B. coli was not cultured in any case. Of the intestinal contents of Billroth II group, 8 cases had no microorganisms but 17 (68.0%) had some, and B. coli was cultured positively in 5 cases (20.0%). And as for the intestinal contents of Billroth I group, 3 had no microorganism, but 4 (57.1%) had some, and B. coli was not detected in any case.

Suture layer: Examination of the serosa suture layer of anastomosis in Billroth II group, revealed 22 cases with no organism, and 3 (13.6%)

with some, but B. coli was not detected in any of the cases. In the serosa suture layer in Billroth I group, 5 had no organism, but 2 had some and they were all broth-positive; no B. coli were found. The kinds and quantity of microorganisms detected in the serosa suture layer in gastroduodenal ulcer cases were less than those in the mucous layer and the latter was nearly equal to those in the gastric and intestinal contents.

The microorganisms cultured from gastric and intestinal contents and each suture layer of anastomosis showed little difference in their kinds and quantity between Billroth I and II groups both in gastric cancer and ulcers. The most remarkable difference was noticed between the cancer and ulcer cases, as previously noticed by Ushigome. The microorganisms were much less in the ulcer than in the cancer cases and the serosa suture layers of anastomosis in the ulcer cases were very close to the aseptic condition.

II. Results of the Bacteriological Examination at the places of Enterostomies

62 cases of surgical intestinal diseases were examined bacteriologically at the place of enterostomy (29 cases of Braun's anastomosis, 10 of enterostomy, 14 of ileo-colostomy and 9 of colo-colostomy). With the exception of Braun's anastomosis, most of them were cases of intestinal stenosis or obstruction and next were cases of tumor and other kinds of surgical intestinal disease. The kinds and quantities of organisms detected are shown in Table I and II.

Intestinal contents

The intestinal contents of the oral and anal sides of anastomosis were examinated separately, but no remarkable difference was seen between them in most cases of all groups. As shown in the Tables, some microorganisms were found in the contents of the upper part of the jejunum at the place of anastomosis and in the small intestine and colon. The percentages of positive cultures of B. coli were 79.3–75.8% in the upper part of the jejunum of Braun's anstomosis cases, 80.0% of enterostomies, 71.4% at the lower part of the small intestine, especially in the ileum, and 100% in the colon. Among them the percentage of cases in which lots of colonies were cultured, was 51.7–55.2% in the upper part of the jejunum, 70.0–80.0% in the small intestine, 71.4% in the ileum and 100-88.9% in the colon. Therefore it is clear that B. coli increases while other organisms decrease towards the lower part of the intestine and colon.

Suture layer

Bacteriological examinations revealed no marked difference in the

TABLE I

Microorganisms Cultured from Various Locations of Intestine, when Anastomosis was Effected

Anatomosis	Location examined	No. of cases examined	Positiv Culture No. of cases (%)	B. coli	Strept. viridans	Strept. hemolyticus	Strept. non hemolyticus	B. alcaligens faecalis	Gram-negativ bacilli	Gram-negativ diplococci	Gram-negativ cocci	Staph. albus	Staph. aureus	Gram-positiv bacilli	Gram-positiv cocci	Gram-positiv diplococci	B. lacticum	B. subtilis	Saccharomyces	B. welchii
Braun's Anastomosis	Oral contents	29	29 (100)	23	1	2	2	2	1	0	0	2	0	1	1	2	2	2	2	3
	Anal contents	29	29 (100)	22	2	3	1	1	1	1	1	2	0	3	1	4	2	3	2	2
	I. suture layer	29	28 (96.5)	20	2	3	1	2	0	1	0	2	0	2	0	3	1	3	1	0
	Serosa suture layer	29	14 (48.2)	11	0	1	0	0	0	0	0	0	0	0	0	1	0	2	1	0
Entero-enterostomy	Oral contents	10	10 (100)	8	0	0	0	0	1	0	0	0	1	1	2	1	1	2	0	0
	Anal contents	10	10 (100)	8	0	0	0	0	1	1	0	0	0	2	3	1	0	2	0	0
	I. suture layer	10	9 (90.0)	6	0	0	0	0	0	0	0	0	1	2	1	1	1	1	0	0
	Serosa suture layer	10	7 (70.0)	4	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0
Ileocolostomy	Oral contents	14	14 (100)	10	0	1	0	0	2	0	0	2	0	3	1	3	1	0	2	2
	Anal contents	14	14 (100)	13	0	1	0	0	2	1	0	2	0	4	1	3	1	0	1	1
	I. suture layer	14	14 (100)	12	0	1	0	0	2	1	0	2	0	3	0	2	1	0	0	1
	II. or III. suture layer	14	12 (85.7)	10	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0
Colocolostomy	Oral contents	9	9 (100)	9	0	0	1	0	0	1	1	0	0	1	0	2	0	0	0	0
	Anal contents	9	9 (100)	9	0	0	0	0	0	1	1	1	0	1	0	1	1	0	0	0
	I. suture layer	9	9 (100)	9	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0
	II. or III. suture layer	9	8 (88.9)	7	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0

quantity and kinds of organisms detected between the mucous suture layer and the intestinal contents at each part. But they were fewer at the serosa suture layers than at the mucous suture layers. The microorganisms in the serosa suture layers were 48.2% of Braun's anastomosis cases, 70.0% of enterostomies, 85.7% of ileo-colostomies and 88.9% of colo-colostomies. The percentages of positive culture of B. coli at the serosa were 37.5% in Braun's anstomosis cases, 40.0% in entero-enterostomies, 71.4% in ileo-colostomies and 55.6% in colo-colostomies. Therefore the aseptic cases at the serosa decreased at the lower part.

III. The Bacteriological Examinations and its Bearing on the Results of Operations

Peritonitis caused by imperfect suture was found in 3 cases, namely in the gastric cancers resected by Billroth I and in 1 case of gastric cancer having colo-colostomy after resection of the stomach and colon transversum. To compare the quantity of organisms detected at the serosa suture layers and the percentage of peritonitis due to imperfect suture, the cases were divided into 1) Plate-positive group, in which many micro-

TABLE II

Quantitive Observations of Microorganisms Cultured from Various Locations of Intestine, when Anastomosis was Effected

Anastomosis	Location examined	No. of cases examined	Number of cases of						
			Cutures			Colonies on plates			
			Sterile	Broth-positiv	Plate-positiv	Scanty	Mod-erate	Fairly abun-dant	Abundant
Braun's anasto-mosis	Oral contents	29	0 (1)	4 (1)	25 (22)	2 (1)	3 (4)	3 (2)	17 (15)
	Anal contents	29	0 (7)	4 (0)	25 (22)	0 (1)	6 (4)	2 (1)	17 (16)
	I. suture layer	29	1 (8)	6 (0)	22 (20)	6 (6)	10 (9)	3 (3)	3 (2)
	II. suture layer	29	15 (3)	6 (3)	8 (8)	3 (3)	4 (4)	1 (1)	1 (1)
Entero-entero-stomy	Oral contents	10	0 (2)	1 (0)	9 (8)	1 (1)	0 (0)	0 (0)	8 (7)
	Anal contents	10	0 (2)	1 (0)	9 (8)	1 (0)	0 (0)	0 (0)	8 (8)
	I. suture layer	10	1 (3)	2 (1)	7 (5)	1 (1)	0 (0)	1 (1)	5 (3)
	II. suture layer	10	3 (3)	2 (1)	5 (3)	1 (0)	2 (1)	0 (0)	2 (2)
Ileocolos-my	Oral contents	14	0 (4)	1 (0)	13 (10)	0 (0)	0 (0)	1 (0)	12 (10)
	Anal contents	14	0 (1)	1 (1)	13 (12)	0 (0)	0 (2)	0 (0)	13 (11)
	I. suture layer	14	0 (2)	1 (0)	13 (12)	0 (2)	4 (1)	3 (3)	6 (6)
	II. or III. suture layer	14	2 (2)	1 (1)	11 (9)	5 (4)	2 (2)	1 (1)	3 (3)
Colocolo-stomy	Oral contents	9	0 (0)	0 (0)	9 (9)	0 (0)	0 (0)	0 (0)	9 (9)
	Anal contents	9	0 (0)	0 (0)	9 (9)	0 (0)	1 (1)	0 (0)	8 (8)
	I. suture layer	9	0 (0)	0 (0)	9 (9)	0 (0)	1 (1)	3 (3)	5 (5)
	II. or III. suture layer	9	1 (2)	3 (2)	5 (3)	2 (2)	3 (3)	0 (0)	0 (0)

organisms were detected and into 2) Aseptic or Broth-positive group in which the organisms were few. The result is that 17 cases of gastro-enterostomy belonged to 1) Group and 72 to 2) Group.

In each group, 1 case of imperfect suture was found. As for bowel cases, 21 of enterostomy or colo-colostomy except those of Braun's anastomosis belonged to the Plate-positive group and 12 to the Aseptic or Broth-positive group. Among them only 1 case of imperfect suture was seen in the Plate-positive group. So, there is no particular relationship between the imperfect suture and quantity of organisms detected at its suture layers. As for the relationship between the kind of organisms and imperfect suture, 1 case of imperfect suture (Gastro-duodenal anastomosis) yielded a positive broth-culture of Streptococcus hemolytics, and another (Gastro-duodenal anastomosis) yielded a positive plate-culture of Gram negative Diplococcus, and 1 case of imperfect suture for colonanastomosis yielded a positive plate-culture of B. coli, but the relation between the kind of organisms and imperfect suture is not so important because there are many cases having these kinds of organisms which recovered favourably.

Summary

1. No remarkable difference was found between Billroth I and II groups concering microorganisms detected in the gastric and duodenal or intestinal contents or from each suture layers of anastomosis after gastric resection in gastric cancer and ulcer.

2. As for the microorganisms cultured from contents at enterostomy and ileo-colostomy, they increased in kinds but decreased in quantity in the upper part of the intestine ,while they became fewer in kind in the lower part of the intestine and B. coli increased towards the lower part B. coli was cultured in all cases from the colic contents.

3. No intimate relation was found between the imperfect suture and the organisms detected at the anastomosis, although 3 cases of suture deficiency were seen in all of the cases and the imperfect suture might be caused by some other factors.

References

1) Ushigome, Tohoku Igaku Zassi, 1949, **40**, 61.
2) Ushigome, Tohoku J. Exp. Med., 1952, **56**, 373.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

Biochemical Studies on Carbohydrates

CLVIII. On Karl Meyer's Mucoitinsulfuric and Hyaluronosulfuric Acid

First Paper

By

Ryosuke Satoh

(佐藤 良助)

(From the Medico-chemical Institute, Tohoku University, Sendai; Director: Prof. H. Masamune)

(Received for publication, May 23, 1952)

With his coworkers Karl Meyer separated in 1937[1] a polysaccharide, containing glucosamine, an uronic and sulfuric acid, by alkaline hydrolysis of commercial gastric mucin, and later on in 1940[2] a polysaccharide of resembling composition from cornea, which diverged from the former in optical rotation and digestibility by the pneumococcus enzyme. He[2] believed the latter polysaccharide to be a monosulfate of "hyaluronic acid," on the glucosamine nature of the hexosamine, its hydrolysableness by the pneumococcus enzyme and optical rotation which "becomes quite similar to that of hyaluronic acid, when calculated on the basis of an ash and sulfate free compound," but did not establish the nature of the uronic acid. Also regarding the former polysaccharide, which he designated "mucoitinsulfuric acid," the uronic acid in it was not strictly identified, whereas glucosamine was separated as hydrochloride.

Lately Wolfrom *et al.*[3] isolated D-glucosaccharic acid after oxidative hydrolysis of the mucoitinsulfate of Meyer. However, only 48 mg. of the saccharic acid were yielded from 1 g. of the material. By their preparation procedure of the acid polysaccharide, an itin and an itinsulfuric acid, if simultaneously existent in one and the same tissue, are separated together and, on the other hand, pig gastric mucosa contains mucoitin (Karl Meyer's hyaluronic acid), as already mentioned by Takahasi and Yasuoka.[4] Therefore the saccharic acid they obtained may have originated in glucuronic acid constituting mucoitin as a contaminant of the preparation.

Thus we undertook re-examination of those polyuronic acids, particularly with concern about their uronic acids. The substances were at first prepared by following Meyer as strictly as possible and further purified by the aid of glacial acetic acid until products were given, that were

Molisch-negative, exhibiting mere vivid Goldschmiedt reaction with α-naphthol and conc. H_2SO_4. Analyses indicated that either of the polyuronic acids was an itinsulfuric acid. The depths of coloration by indole and orcinol of the cornea polysaccharide coincided with each other as expressed as glucuronic acid, but, contrariwise, those of the gastric polysaccharide agreed as expressed as galacturonic acid rather than as glucuronic acid. The developing colours by those reagents from mannuronic acid is characterized with green fluorescence, which was not noticed in the reactions of the preparations. They rotated D-light almost similar, $[\alpha]_D$'s being approximately $-25°$. In short, one of the claims of Meyer appeared to be acceptable as real that the cornea polysaccharide is monosulfuric acid ester of hyaluronic acid, but the other polysaccharide to be in need of a scrutiny. Our preparation of the cornea substance diverged from Meyer's in the magnitude of negative optical rotation, which, however, the writer thinks, can not be an evidence against the identity of the carbohydrate skeleton of this polysaccharide and hyaluronic acid.

Thanks to a new devise of Masamune and Maki,[5] not only hexosamines but also hexuronic acids have become sharp-separable between themselves by paper partition chromatography. So, the writer hydrolyzed the stomach substance under various conditions to subject to that chromatography. Diagrams were obtained with spots of glucosamine and galacturonic and glucuronic acid besides that corresponding to the position common to the basic disaccharide units of hexosamine-containing polyuronic acids, which are generally difficult to completely hydrolyze without decomposition. The spot of glucuronic acid was comparatively weak and looked due to mucoitin separated together with the itinsulfuric acid in view. Moreover, the writer prepared the basic disaccharide of the polysaccharide and desaminized it. The hydrolysate of this desamination product gave a spot of galacturonic acid but none of glucuronic acid. Most probably the mucoitin that was contained in the polysaccharide preparation was so little that mucosin neither amounted beyond a trace nor was present at all in the unit disaccharide preparation.

Needless to say, Levene applied the name mucoitinsulfuric acid to acid polysaccharides composed of acetylglucosamine, glucuronic acid and sulfuric acid in the moleculr proportions of 1:1:1, irrespective of their structure. Therefore, the itinsulfuric acid in the gastric mucin, whose uronic acid component is galacturonic acid, does not conform to his terminology of mucoitinsulfuric acid, whereas the one in cornea does. The writer proposes to call the former "gastroitinsulfuric acid" and the latter mucoitinsulfuric acid. That "hyaluronic acid" is deemed to possess a structure same as the carbohydrate skeleton of the cornea sub-

stance gives a favourable argument for the denomination of " mucoitin " we applied to it.

Experimental

The itinsulfuric acids in problem were prepared according to Meyer and others[1][2] with some unavoidable modifications and purified.

I. Cornea Itinsulfuric Acid

164 fresh ox corneas were cut into smallest possible pieces with scissors. The total weight amounted to 87 g. They were mixed with 500 cc. of 50% urea, alkalized to pH 9.0 with NaOH (1 *N*) and stood for 3 weeks at room temperature, with occasional agitation and adjustment to the original alkalinity. Centrifuged. The centrifugate was again extracted for 1 week with 250 cc. of the urea solution and centrifuged. The supernatant fluids were combined, diluted four times and acidified to pH 3.8 with acetic acid (20%), whereby precipitation occurred. The precipitate centrifuged was washed with four 20 cc. portions of water, taken up in 120 cc. of 10% $CaCl_2$ and after alkalinizing to pH 7.4, shaken. The turbid solution given was then shaken with 32 cc. of a chloroform-amylalcohol (3:1 by volume) mixture for 15 hours on an apparatus. The centrifuged upper layer (translucent) was again treated with 16 cc. of the same organic mixture for 10 hours. Centrifugation gave this time a transparent solution. This was precipitated with 2 volumes of abs. alcohol and a little anhydrous sodium acetate, washed well with abs. alcohol and thereafter dissolved in 20 cc. of water by the aid of 0.3 cc. of 1/5 *N* NaOH. The solution (pH 7.0) was mixed with an equal volume of 2 *N* acetic acid and vigorously shaken with 2 g. of purified kaolin for 1 hour and the centrifugate stirred up with 5 cc. of 1 *N* acetic acid and again centrifuged. The successive supernatant fluids (opalescent) were further filtered to eliminate floating particles, and 2 volumes of abs. alcohol and a little abs. alcohol saturated with anhydrous sodium acetate added. Then the deposit was washed thoroughly with abs. alcohol and taken up in 3 cc. of water, and the insoluble part centrifuged and washed with 2 cc. of water. The solution and washing were precipitated with 20 cc. of glacial acetic acid and a little anhydrous sodium acetate, washed with abs. alcohol repeatedly to free from acetic acid as far as possible and with acetone and dried in a desiccator. 120 mg. of a white powder that corresponded to Meyer's preparations were thus yielded. Since the product here gave the positive Molisch reaction besides the positive Goldschmiedt, purification was processed as follows.

It was dissolved in 10 cc. of water, a small insoluble part rejected by centrifugation and the clear supernatant mixed with 30 cc. of glacial

acetic acid. On addition of anhydrous sodium acetate to the opalescent solution, an abundant flocculent precipitate occurred. This was further on reprecipitated in a similar manner more than ten times. The substance took more and more glassy appearance (translucent jellylike form) and showed less and less positive and finally negative Molisch reaction. Thereupon it was washed with alcohol several times to remove acetic acid completely and dried. Furthermore it was liberated from an insoluble contaminant after taking up in 3 cc. of water, precipitated and washed with alcohol and dried. Crop, 59 mg. Goldschmiedt strongly positive, biuret negative.

For the measurement below the substance was dried to constant at 100–110°C under reduced pressure.

Analysis. See Table I.

TABLE I

Composition of the Cornea Polysaccharide

Analysis	In per cent	In equivalents per equivalent weight
N	3.0	1.1
Glucosamine*	33.2	1.0
Hexuronic acid†		
i) as glucuronic acid‡		
Indole reaction	34.2	
Orcinol r.§	34.7	
Mean	34.5	1.0
ii) as galacturonic acid//		
Indole r.	18.6	
Orcinol r.§	40.3	
Acetyl¶	10.5	1.3
Hydrolysable sulfur**	7.0	1.2
Iodine use as glucose††	1.8	
Ash‡‡	16.8	

* Hamasato & Akakura method. † The orange colours developed by mannuronic acid with the orcinol and indole reagents are accompanied with green fluorescence which impairs the colorimetry against the standard prepared with glucose. That fluorescence did not occur here. ‡ Cf. Hisamura[6] and Masamune & Hisamura.[7] § Cf. Ozaki.[8] // Cf. Masamune, Suzuki & Kondoh.[9] ¶ Suzuki's modification of Friedrich, Rapoport & Sternberg method.[10] ** Suzuki method.[10] †† Macleod & Robison method. ‡‡ Pregl method without the use of H_2SO_4.

Optical rotation in water. $[\alpha]_D^{13}=\frac{-0.35\times 100}{1.38\times 1}=-25.4°.$

II. The Itinsulfuric Acid in Pig Stomach Mucosa

Preparation procedure

Since the commercial "gastric mucin" was inaccessible, it was first

prepared by the method briefly described by Meyer *et al.*[1]:—Gastric mucosa was torn off together with the mucus from 15 fresh pig stomachs and masticated (total weight 6120 g.), then it was kneaded with 200 cc. of water and enough 10% HCl to shift the pH to 2.0, and after addition of 40 cc. toluene and 40 cc. chloroform, placed in a thermostat at 37°C. Additional hydrochloric acid was added from time to time to maintain the mixture at the incipient acidity. After 10 days the digestate (a translucent yellow-brown fluid) was separated from the non-digestible residue and 4 volumes of alcohol added. And the white gummous precipitate was centrifuged, washed well with alcohol and dried in a desiccator over $CaCl_2$. 105 g. of a non-sticky powder were obtained.

The material thus obtained was agitated in 1 *l.* of 1 *N* NaOH and stood for 2 days at room temperature (15–19°C). Then, the brown solution containing insoluble particles was shaken with 20 cc. of toluene and centrifuged, whereby the mixture separated into three layers. The middle transparent layer was transferred to a beaker and 2 volumes of alcohol added. The slimy precipitate was washed with alcohol several times and a sticky clump was given, which was dissolved in 50 cc. of water, precipitated with 10 volumes of glacial acetic acid and washed with alcohol exhaustively and with acetone. 9.5 g. was the brown heavy powder given here. This was dissolved in 500 cc. of water and neutralized with 10% NaOH (4.2 cc. used), and followingly 1 *N* HCl (56.02 cc.) added up to 0.1 *N*. Vigorously shaken with 50 g. of kaolin and filtered. The bulk on the funnel was shaken with 50 cc. of water and again filtered. And the filtrates were precipitated with 4 volumes of alcohol and after washing with alcohol twice, extracted with two 30 cc. portions and one 10 cc. portion of water. The combined solutions (70 cc.) were precipitated with 350 cc. (5 volumes) of alcohol and anhydrous sodium acetate, washed many times with alcohol and dried. The product (a yellowish powder) at this stage, which corresponded to Meyer's preparations, amounted to 4.8 g. Almost biuret-negative but strongly Molisch-positive. Neuberg & Saneyoshi test for hexuronic acid resulted in positive.

Purification. It was dissolved in 500 cc. of water and 10 g. of heated charcoal were added, while hot (70–80°C), under agitation. The mixture was further stirred up for 30 minutes and filtered with suction. The almost colorless filtrate was distilled in vacuo to 30 cc. 150 cc. of alcohol were added and the precipitate was centrifuged, washed with alcohol and dried. The substance diminished to 2.6 g. and its Molisch reaction became less strong. Repeating treatments with glacial acetic acid were next effected:—

1) It was taken up in 100 cc. of water and 400 cc. of glacial acetic acid were added in small portions. The precipitate was subjected to a

similar process again and again, using later NaCl, in addition, to complete the precipitation (Without NaCl-addition the centrifuged supernatant remained turbid). The precipitate after more than 20 repetitions was washed with many changes of alcohol and dried. 1.2 g. of a white powder, showing only faint Molisch reaction, were given.

2) This was freed from some insoluble after shaking with 50 cc. of water and 4 volumes of glacial acetic acid and some NaCl added. The centrifugate was likewise reprecipitated from water scores of times. The consecutive precipitates changed more and more glassy, but a trace of a Molisch-giving impurity could not be eliminated. The final precipitate was again washed with alcohol and dried.

3) The product in 2) (a white powder, 900 mg.) was dissolved in 50 cc. of water (A minute insoluble part was not discarded), the pH moved to 7.4 with 5% Na_2CO_3 and after addition of 1 cc. of formalin, the Na_2CO_3 solution added again to re-elevate the pH to 7.4. The mixture was stood for 1 hour at room temperature and precipitated with 5 volumes of alcohol with subsequent washing with alcohol. The powder dried was again taken up in 50 cc. of water, the insoluble part rejected by centrifugation and 4 volumes of glacial acetic acid added. The dissolution and precipitation were further carried on many times with addition, when necessary, of NaCl besides glacial acetic acid. At last the substance in water showed no turbidity at all on addition of glacial acetic acid alone but came out in flakes on adding NaCl in addition. Scores of reprecipitations were again conducted. Thereafter, the transparent jellylike deposit was washed with abs. alcohol exhaustively and after drying once, carried down from a small volume of water with abs. alcohol and a little NaCl. The present deposit was also washed with abs. alcohol repeatedly and dried—**Prep. I,** a white powder weighing 520 mg.

In another similar run started with 40 g. mucin (from 2460 g. fresh mucosa), 120 mg. of **Prep. II** were produced (The intermediate product corresponding to Meyer's preparations amounted to 2.0 g.).

Chemical and physical properties

Qualitative tests. Preps. I and II resembled each other in chemical qualitative respects. Biuret and Molisch negative; Goldschmiedt, Neuberg & Saneyoshi and aniline acetate test distinctly positive. The indirect Osaki & Turumi reaction resulted in positive but not the direct.

Quantitative analysis. For the analysis, the preparations were dried to constant at 100–110°C under low pressure. The compositions are illustrated in Table II. To calculate out galacturonic acid from the furfural yields, the latter were multiplied with a factor 10.36, which was obtained by treating 1–4 mg. of barium galacturonate (Ba found 25.9%;

TABLE II

Composition of the Stomach Itinsulfuric Acid

Analysis	In per cent		In equivalents per equivalent weight	
	Prep. I	Prep. II	Prep. I	Prep. II
N	2.4	2.7	1.0	1.1
Glucosamine	31.6	32.7	1.0	1.0
Hexuronic acid				
i) as glucuronic acid				
Indole reaction	67.0	70.2		
Orcinol r.	34.4	36.8		
ii) as galacturonic acid				
Indole r.	34.4	36.8		
Orcinol r.	36.1	38.6		
Mean	35.3	37.7	1.0	1.1
Calc. fr. furfural yield*		39.2		1.1
Acetyl	8.8	10.0	1.1	1.3
Hydrolysable sulfur	5.5	5.7	0.97	0.97
Iodine use as glucose	1.9	2.0		
Ash	15.7	16.5		

All the analytical methods employed were the same as those in Table I, exclusive of the one to measure the furfural production. * Youngburg method[11]; also see the text.

calc., 26.2%) according to Youngburg method.[11] Within those limits of amount taken, the galacturonate gave almost coinciding furfural yields per mg.

Optical rotation in water

$$\text{Prep. I, } [\alpha]_D^{20}=\frac{-0.35\times 100}{1.56\times 1}=-22.4°.$$

$$\text{Prep. II, } [\alpha]_D^{20}=\frac{-0.56\times 100}{1.80\times 1}=-31.1°.$$

Paper partition chromatography (The writer is indebted to Mr. M. Maki). Another batch of the stomach itinsulfuric acid was produced similar to above for the chromatographic investigation.

1) At first the preparation was hydrolyzed under various conditions with sulfuric acid, subjecting mucoitin (barium salt) and chondroitinsulfuric acid (acid sodium calcium salt) to a parallel run for comparison, which had been prepared after Isikawa[12] and Jorpes respectively. The hydrolysates were removed from most of sulfuric acid with baryta, and evaporated in vacuo to a small volume for putting on paper strips. The volumes and concentrations of sulfuric acid used, temperatures and durations of time for hydrolysis, the volume of the condensates and else are tabulated as follows:

Method No.	Polysaccharide: name*	Polysaccharide: amount taken (mg.)	Hydrolysis: agent: sulfuric acid: conc.	Hydrolysis: agent: sulfuric acid: vol. cc.	Hydrolysis: temperature C	Hydrolysis: duration (hrs.)	Dilution of the hydrolysates with water to precip. out H_2SO_4	Condensate: vol. cc.	Condensate: taken for irrig. cc.
I	M C G	100	1 *N*	10	100°	6	2 times	0.5	0.02
II	M G	"	5 *N*	"	37°	82	10 times	"	"
III	M C G	"	10 *N*	"	"	"	20 times	"	"
IV	M C G	"	5 vol. of H_2SO_4 (sp. gr. 1.84)+ 2 vol. of H_2O	0.7	in an ice chest (0 ~6°)	96	to 100 cc.	"	"

* M=mucoitin (acid barium salt), C=chondroitinsulfuric acid (acid sodium calcium salt), G=gastroitinsulfuric acid (acid sodium salt).

The solvent used for irrigation, amylalcohol-pyridine-water (7:7:6). Solvent fronts run 40 cm. Temperature 30°C. Duration of test 23 hours. Developers, aniline-phthalic acid of Partridge and 0.2% ninhydrin in butanol. In Fig. 1 (e-t) are diagrammatically reproduced the chromatograms obtained. All of the native polysaccharides were found to have not been completely hydrolyzed, showing a spot of the residual of respective disaccharide units. The different basic disaccharides had Rf values practically one and the same (Rf=0.025), which did not vary whether the disaccharides were in a salt form or free. Mucosin- and chondrosin-sulfate once separated were also run (1 and 2 in Fig. 1). The scission of the stomach itinsulfuric acid and mucoitin hardly reached beyond the disaccharide stage under the conditions of Method I, but, by the more drastic procedures (Methods II-IV) the monosaccharide components were liberated sufficiently to give distinct spots demonstrating the presence of galacturonic acid and glucosamine (aniline-phthalic acid as well as ninhydrin positive) and a small amount of glucuronic acid (weak spots) in the preparation of the stomach itinsulfuric acid, and glucuronic acid and glucosamine in mucoitin. Chondrosin, the basic disaccharide of chondroitinsulfuric acid, proved extremely resistant to hydrolysis under the conditions of all of those methods.

2) To ensure that the itinsulfuric acid in question contains galacturonic acid, its basic disaccharide (" gastrosin ") was isolated by the procedure Levene had employed in obtaining chondrosin and further desaminized to make the uronide union less stable (Cf. former works[14] on chondrosin and mucosin published from our Institute):—

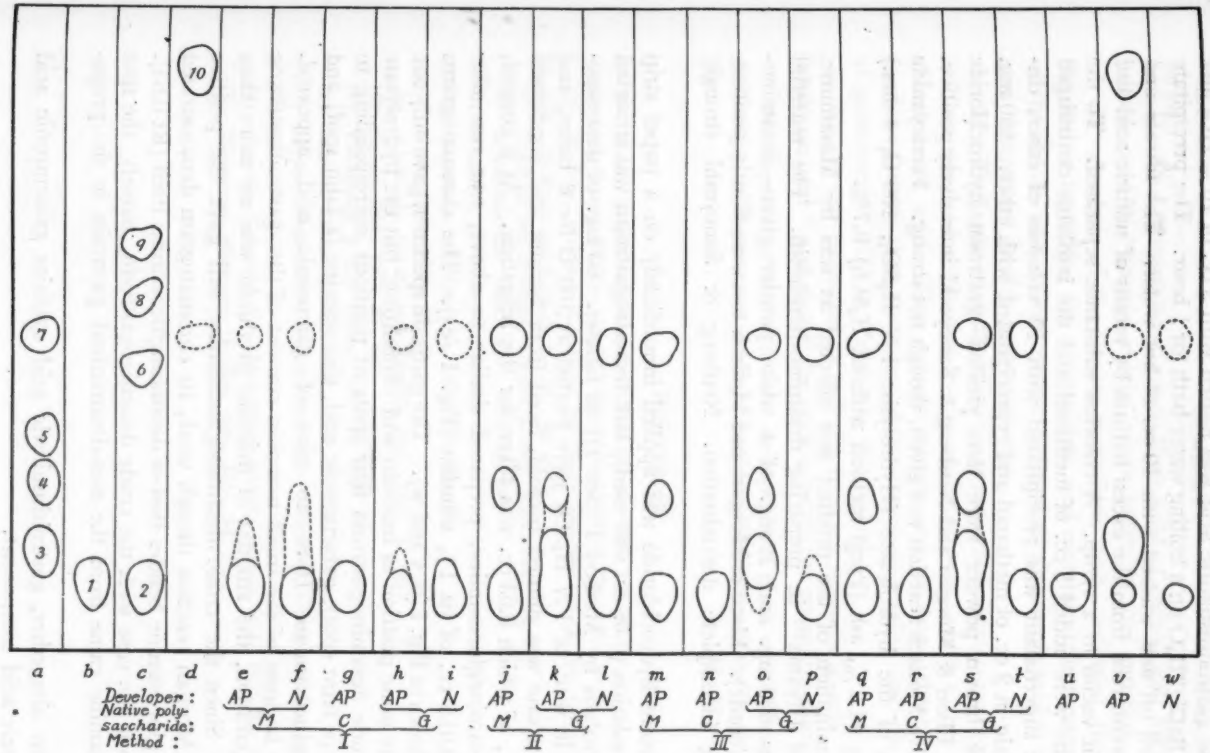

10
9
8
7
6
5
4
3
2
1
a b c d e f g h i j k l m n o p q r s t u v w
Developer : AP N AP AP N AP AP N AP AP AP N AP AP AP N AP AP N
Native polysaccharide: M C G M G M C G M C G
Method : I II III IV

Fig. 1. Amylalcohol-pyridine-water (7:7:6) chromatograms.

a-d: standards, e-t: the native acid polysaccharides, u: the basic disaccharide unit of gastroitinsulfuric acid (gastrosin) as hydrochloride, v-w: desaminogastrosin, AP: developed with aniline-phthalic acid, N: developed with ninhydrin, M: mucoitin, C: chondroitinsulfuric acid, G: gastroitinsulfuric acid, 1: mucosin sulfate, 2: chondrosin sulfate, 3: galacturonic acid, 4: glucuronic acid, 5: mannuronic acid, 6: chondrosamine hydrochloride, 7: glucosamine hydrochloride, 8: galactose, 9: mannose, 10: desaminoglucosamine (Rf. 0.65). (a-d: a 0.015 cc. portion of a solution, which was 1% with respect to each individual sugar, was put on a paper strip).

1 g. of gastroitinsulfuric acid was heated with 3 cc. of 10% HCl and 0.47 g. of $BaCl_2 \cdot 2H_2O$ on a boiling water-bath for 1 hour. The precipitate was filtered off and washed with 10 cc. of hot water. The filtrate and washing were freed from the excess barium by means of sulfuric acid and distilled in vacuo to a syrup. A colorless substance separated. To the still residue were added 3 cc. of methanol and the insoluble centrifuged off. The supernatant was precipitated with 10 volumes of ether, dissolved again in 2 cc. of methanol and reprecipitated with ether. 650 mg. of a dark brown powder were thus yielded—gastrosin hydrochloride (crude). Elson & Morgan and Neuberg & Saneyoshi intensively positive. The positive Molisch reaction was given, though not strong. Ferricyanide reduction of the hydrolysate (Hydrolysis: 1 *N* H_2SO_4, 100°C, 4 hrs.) as glucose 41.2%, ash (Pregl method without H_2SO_4) 9.7%.

Desamination of the product was effected as was by Masamune, Maki and Hiyama[15] in preparing desaminochondrosin. The material taken was 600 mg. and 72 mg. of a white powder given—desaminogastrosin (crude). Elson & Morgan and Molisch test were faintly positive, indicating incomplete desamination. Neuberg & Saneyoshi strongly positive.

Gastrosin hydrochloride was applied immediately on a paper strip as a 1% solution (0.03 cc. was used), but desaminogastrosin was irriagted after hydrolysis by Method I (See 1)) as follows. 60 mg. of desaminogastrosin in 6 cc. of 1 *N* H_2SO_4 were heated at 100°C for 6 hours, and the hydrolysate was diluted twofold, freed from barium and condensed to 0.5 cc., of which 0.03 cc. was taken for the irrigation. As a control, crude desaminoglucosamine, prepared similar to above, was run after putting 0.015 cc. of its 1% solution (Fig. 1 (d)). The chromatograms are also shown in Fig. 1 (u, v and w). The gastrosin specimen gave only one spot at the same position as mucosin and chondrosin, but the hydrolysate of the crude desaminogastrosin four spots at positions corresponding to gastrosin (a faint spot), galacturonic acid, glucosamine (a faint spot) and desaminoglucosamine. Here no spot of glucuronic acid appeared. Probably because of the small mucoitin content of the gastroitinsulfuric acid preparation, the amount of mucosin produced was no more than a trace. Since the crude desaminoglucosamine still gave the positive Elson & Morgan reaction though weak, its chromatogram demonstrated a spot of glucosamine besides that of desaminoglucosamine itself (Rf 0.65). Similar was the case with the crude desaminogastrosin, namely, the spot of glucosamine came from the non-desaminized gastrosin in the preparation.

Taken altogether, gastroitinsulfuric acid contains galacturonic acid as its uronic acid component.

Summary

1) Karl Meyer's hyaluronosulfuric acid of cornea and his mucoitinsulfuric acid of "gastric mucin" were prepared and purified.

2) Analysis of the former itinsulfuric acid confirmed the result of the author regarding the composition, and moreover the figures obtained by the indole- and orcinol-method pointed to glucuronic acid as the uronic acid component in this polysaccharide.

3) The latter itinsulfuric acid was suspected not to contain glucuronic acid on rough identification by the analytical way, which rather suggested galacturonic acid. This polysaccharide and its basic disaccharide desaminized were then investigated by chromatography, and the presence of galacturonic acid in them was established.

4) It was proposed to call the stomach itinsulfuric acid "gastroitinsulfuric acid" and to call the cornea substance mucoitinsulfuric acid.

The expenses of this work were defrayed by a grant which was given from the Education Department through the Grant Committee of the Science Council.

H. Masamune.

References

1) Meyer, Smyth and Palmer, J. Biol. Chem., 1937, **119**, 73.
2) Meyer and Chaffee, Am. J. Ophthalmol., 1940, **23**, 1320.
3) Wolfrom, Weisblat, Karabinos, Mc Neely and McLean, J. Am. Chem. Soc., 1943, **65**, 2077; Wolfrom and Rice, ibid., 1947, **68**, 1833.
4) Takahasi and Yasuoka, Tohoku J. Exp. Med., 1947, **49**, 65.
5) Masamune and Maki, ibid., 1952, **55**, 299.
6) Hisamura, J. Bioch., 1937, **26**, 359.
7) Masamune and Hisamura, ibid., 1938, **28**, 137.
8) Ozaki, ibid., 1936, **24**, 77.
9) Masamune, Suzuki and Kondoh, ibid., 1940, **31**, 347.
10) Suzuki, ibid., 1938, **27**, 367.
11) Youngburg, J. Biol. Chem., 1927, **73**, 599.
12) Isikawa, Tohoku J. Exp. Med., 1951, **53**, 217.
13) Levene, Hexosamines and mucoproteins, Longmans, Green and Co. London, 1925 p. 110.
14) Nagaoka, Tohoku J. Exp. Med., 1950, **53**, 29; Isikawa, ibid., 1951, **55**, 23.
15) Masamune, Maki and Hiyama, ibid., 1951, **54**, 315.

The Tohoku Journal of Experimental Medicine, Vol. 56, No. 4, 1952

On the Mechanism of the Increase of Blood Non-Protein Nitrogen in Eclampsia, Convulsion etc.*

By

Tatsuo Suzuki
(鈴木 辰夫)

(From the Department of Obstetrics and Gynecology, Fukushima Women's Medical College, Fukushima; Director: Prof. K. Kushima)

(Received for publication, May 24, 1952)

Although many disputes have been made regarding the quantitative behaviour of non-protein nitrogen of blood (N.P.N.) in eclampsia patients, it is now generally believed to increase. Also the increase of N.P.N. after delivery and operation has been reported by many authers. With respect to the mechanism of N.P.N. increase, Amano[1)2)] suggested that it is probably due to the stimulation of some cerebral center controlling the protein metabolism, because he found in dogs no increase of N.P.N. after convulsion by strychninum nitricum in contrast with a marked increase after that by picrotoxin. Akiyama[3)] inferred that the rise of N.P.N. in eclampsia is partially caused by the renal insufficiencies and muscular contraction, but mostly by some "convulsion toxin" which acts on the cerebral center of the protein metabolism. Eufinger[4)] put forward also similar opinion that the amount of N.P.N. is raised after eclampsia convulsions by an abnormal metabolic product rather than by the renal insufficiency. To acquire more accurate knowledge in this connection the experiments regarding N.P.N. after operation, delivery and convulsion have been undertaken which are dealt with in this account.

Experiments and Discussion

N.P.N. Increase due to the Eclampsia Convulsion, Delivery and Operation

At first, N.P.N. was estimated before and after eclampsia convulsion in 3 cases. N.P.N. increased slightly (+4.2—+5.6 mg/dl) as had been observed by Wada.[5)]

* Read before the 2nd meeting of the Japanese Obstetrical and Gynecological Society, April, 1950.

Further, in 4 cases of artifical abortion (5 to 7 months pregn.) and 2 cases of normal labor, the amount of N.P.N. was seen to rise after delivery by +4.3 to +12.2 mg/dl, the average being +10.5 mg./dl, in accord with the results of Hohlweg,[6] Gibuns, Shinoda,[7] Nakai,[8] Kuji, Wada[5] and others.

But when phenobarbital, a hypnotic acting on the cerebral trunk, (0.15 to 0.2 g. a day) was given before as well as after delivery, N.P.N. showed almost no change (−2.0 to −1.7 mg/dl) in 4 cases of artifical abortion and in one of 2 cases of normal labor. In the remaining one of normal labor, N.P.N. increased only slightly (+1.2 mg/dl) in 3 hours after delivery. In this case the administration of phenobarbital was discontinued just after labor, and N.P.N. increased distinctly (+8.3 mg/dl) after 34 hours from delivery, but after subsequent re-administration of phenobarbital it decreased within 24 hours to a value+2.2 mg/dl above the one before delivery.

As to N.P.N. after operation, Nakayama[9] and many other investigators reported that it begins to increase markedly soon after the operation, and after having reached the maximum in 2 to 3 days, becomes reduced to the normal in 7 days. In my 5 cases (Doléris op., panhysterectomy, excision of Fallopian tubes, abdominal cesarean section and supravaginal amputation of uterus) there occurred an increase of +5.4 to +14.1 mg/dl, +9.6 mg/dl on an average, but almost none (−1.3 to +1.9 mg/dl), when phenobarbital (0.2 g. a day) was given. In a case of abdominal cesarean section, in which phenobarbital was not given after the operation, it showed a high value on the second day and on administering the drug again on the third day, returned to the preoperative value.

From the above findings it might be said that N.P.N. increases after all of eclampsia convulsion, labor and operation, and that the increase in labor and operation at least is ascribable to the excitement of the cerebral trunk. Moreover, to study the mechanism of N.P.N. increase the writer carried out the following experiments:—

1. A 10% solution of cardiazol, a convulsion drug acting on the cerebral trunk, (0.2 to 0.3 cc.) was intravenously injected to rabbits. 7 animals (weighing over 2 kg.) showed clonic, tonic convulsion and N.P.N. increased distinctly (+7.2 to +17.2 mg/dl, average +14.4 mg/dl), but in 3 animals, which exhibited no convulsion despite of almost the same dose of cardiazol, no change of N.P.N. (−0.2 to +1.1 mg/dl) happened.

2. 8 rabbits were intravenously injected a 0.1% strychinine solution, a peripheral convulsion drug, (0.3 to 0.4 cc.) to produce tonic, clonic convulsion. By this convulsion N.P.N. was also raised though little (+0.2 to +14.0 mg/dl, average +6.4 mg/dl), disagreeing with the result of Amano, who said that there is no increase after convulsion caused by

strychinine in dogs. In the same rabbits, 4 or 5 days later, convulsion was induced with strychinine (0.3 to 0.6 cc.) again after 30 minutes from preceding subcutaneous injection of 0.5 cc. of 10% phenobarbital, entailing almost no change of N.P.N. (−3.6 to +6.4 mg/dl, average +0.3 mg/dl). Since subcutaneous injection of mere phenobarbital does not alter the value of N.P.N., the N.P.N. increase by strychinine convulsion is mostly ascribed to the excitation of cerebral center.

To decide whether this center is localized in cortex or trunk, strychnine convulsion was induced in 5 animals which had been subcutaneously injected 30 minutes before with 0.2 to 0.3 g. chloratum hydratum, a cortical hypnotic. Hereby greater increase (+10.7 to +20.0 mg/dl, +14.8 mg/dl on an average) of N.P.N. took place than when strychnine alone was used. The rise of N.P.N. after strychnine convulsion thus looked to spring mostly in the shock of the cerebral trunk. So, in order to find in which part of the cerebral trunk the controlling center of N.P.N. is situated, an attempt was made to cause strychnine convulsion after having poured some anesthetics in hypothalamus, where the muclei of autonomic nerves gather. Namely, taking 3 rabbits to the end in view, a very small hole was made in the skull which allowed a needle to arrive at nuclei hypothalamicus ventromedialis and hypothalamicus lateralis. The convulsion was given rise to in 15 minutes after pouring a drop of 10% phenobarbital either in nucleus hypothalamicus ventromedialis or in nucleus hypothalamicus lateralis. N.P.N. increased by +3.3 mg/dl on an average (−1.5 to +9 mg/dl) in the former case, and by +3.8 mg/dl on an average (+1.6 to +7.5 mg/dl) in the latter. The elevations reached only half of the height in the convulsion by mere strychnine (+6.4 mg/dl on an average), offering a proof that phenobarbital prevents hypothalamus from excitation. The inhibitory effect of phenobarbital here was not so distinct as when the drug was injected subcutaneously. This was probably because hypothalamus on the opposite side was not soaked with phenobarbital within so short time as 15 minutes, the interval till applying strychinine, and, hence a similar experiment was repeated moreover on 5 rabbits after prior treatment with 3% procainum hydrochloricum, without resultant increase of N.P.N. (when nucleus hypothalamicus ventromedialis was anesthetized −1.5 to +0.5 mg/dl, average −0.3 mg/dl; when nucleus hypothalamicus lateralis was anesthetized, −3.8 to +4.5 mg/dl, average +0.4 mg/dl) as expected. In these cases the minimum dose of strychnine requisite for developing convulsion was 0.53 cc. in averages, far greater than the mean minimum 0.3 cc. in the case with strychnine alone, and also greater that those after subcutaneous injection of 10% phenobarbital solution (0.45 cc.) and of chloratum hydratum (0.42 cc.).

Judging from what were observed, it is regarded as justified to suspect

that, in regulation of N.P.N. amount, cerebral trunk, particularly hypothalamus plays the most cardinal part.

Change in N.P.N. due to Direct Hypothalamus Invasion

Nucleus hypothalamicus ventromedialis or lateralis was punctured with a needle, employing 3 rabbits each, but the N.P.N. did not undergo any noticeable change (in the former case −0.7 to +3.8 mg/dl, mean +1.0 mg/dl and in the latter case −0.4 to +1.7 mg/dl, mean +0.7 mg/dl).

Next, the writer estimated the change of N.P.N. 15 minutes after giving a drop of Hinterin (posterior pituitary hormone), which is conceded to bear close relation with eclampsia, in hypothalamus in a similar manner. No increase of N.P.N. was found (−0.7 to +1.8 mg/dl, average −0.1 mg/dl) in 3 cases when nucleus hypothalamicus ventromedialis was reached by the hormone, but a slight increase (−3.8 to +7.5 mg/dl, average +2.1 mg/dl) in 5 cases when nucleus hypothalamicus lateralis was reached.

In the third, a drop of a 0.1% acetylcholine solution was applied to the hypothalamus as above and N.P.N. was determined 15 minutes after. When nucleus hypothalamicus ventromedialis was reached by the agent, there was even a slight decrease of it (+4.5 to −8 mg/dl, average −5.8 mg/dl), but a slight increase (+3.0 to +6.0 mg/dl, average +4.6 mg/dl) in all cases when nucleus hypothalamicus lateralis was reached. Namely, the N.P.N. increase is considered to ensue from the excitement of nucleus hypothalamicus lateralis.

In short, it is concluded that N.P.N. increases after operation, labor and convulsion by the stimulation of diencephalon, in particular of hypothalamus.

Cobb-Bailey,[10] Go,[11] Iwata,[12] Tsukahara[13] and others considered corpora quadrigemina of diencephalon to be the convulsion center, since Sherrington had mentioned that it is the most important part of the brain. Later Takahashi[14] successfully demonstrated an epilepsy-like convulsion by heating corpora quadrigemina, and Miyoshi[15] also considered the epilepsy convulsion to be due to the excitement of corpora quadrigemina. And at present many authors are of the opinion that the convulsion center exsists in the cerebral trunk. As eclampsia convulsion closely resembles epilepsy convulsion, the former must also have a bearing on cerebral trunk. Consequently my experimental deduction that the control center of N.P.N. lies in hypothalamus especially in nucleus hypothalamicus lateralis and its neighbourhood supports the opinion of Kushima[16] and coworkers who pointed to the cerebral trunk, and, among other parts, the hypothalamus as the center of eclampsia convulsion.

Summary

1. Blood non-protein nitrogen (N.P.N.) increases after the convulsion in eclampsia, delivery and operation.

2. a) The rise of N.P.N. after delivery and operation is prevented by phenobarbital.

b) N.P.N. rises in rabbits remarkably after convulsion by cardiazol but only slightly after convulsion induced by strychnine. And in the latter case, no increase of N.P.N. is noticed at all when the rabbits are treated in advance with phenobarbital.

c) If rabbits are treated before the strychinine convulsion with chloratum hydratum instead of phenobarbital, N.P.N. -increase is not inhibited, but rather accelerated. To sum up, the behaviour of N.P.N. is mostly controlled by a cerebral center and this center is localized in the cerebral trunk.

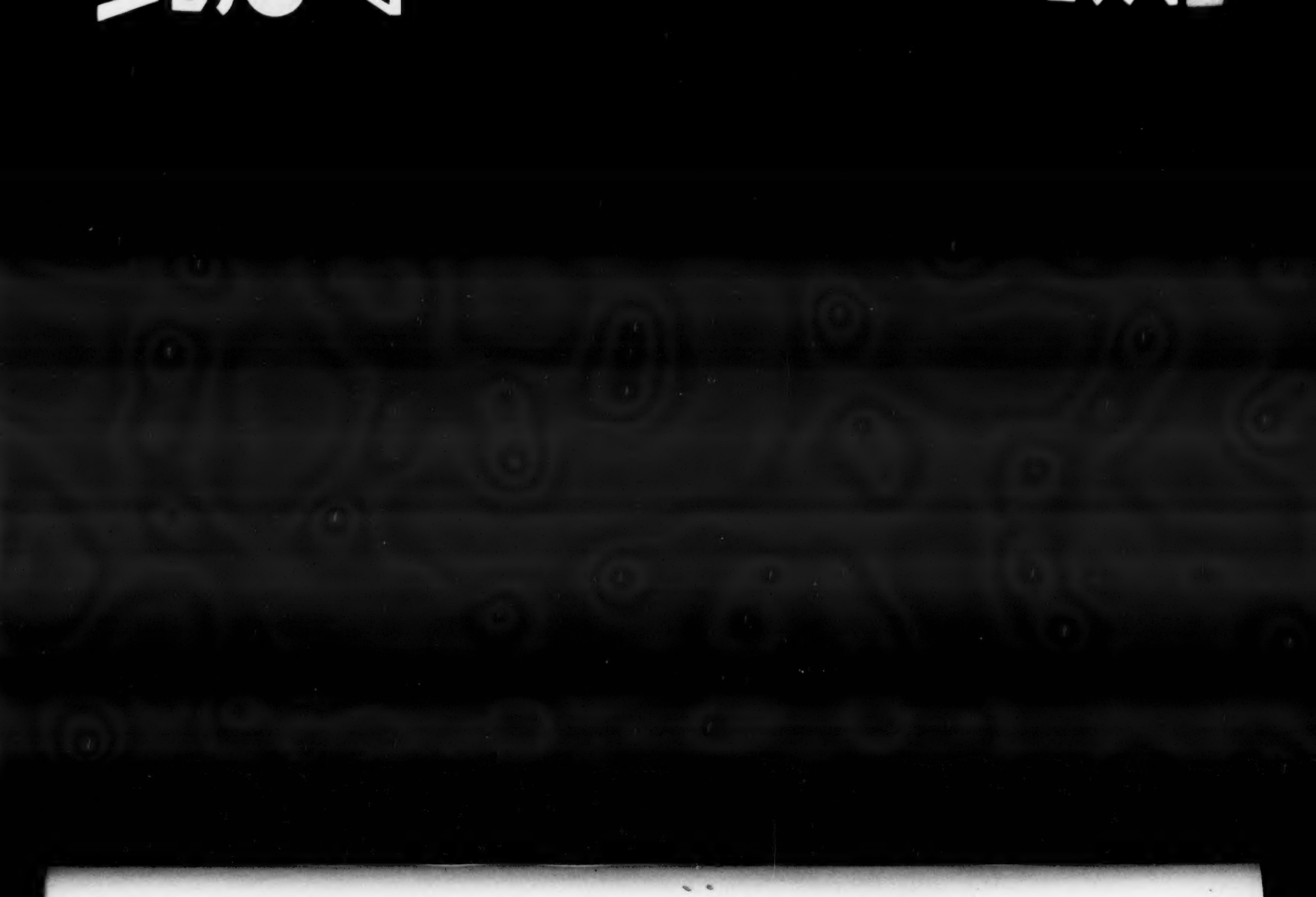
3. Increase of N.P.N. hardly takes place when convulsion is caused by strychnine after previous treatment with phenobarbital or procainum hydrochloricum of hypothalamus, especially of nucleus hypothalamicus ventromedialis or lateralis, in other words, the center in question is most probably in hypothalamus.

4. When posterior pituitary hormone (Hinterin) or acetylcholine is dropped in nucleus hypothalamicus ventromedialis, no change in N.P.N. is observed. However, if nucleus hypothalamicus lateralis is stimulated in the same manner, N.P.N. increases though slight. Accordingly, the controlling center of N.P.N. must be sought in nucleus hypothalamicus lateralis and its neighbourhood.

5. The two facts that the controlling center of N.P.N. lies in hypothalamus and that N.P.N. increases after eclampsia convulsion give a support to the claim of Kushima that the center of eclampsia convulsion is situated in hypothalamus.

I am very grateful to both Profs. Shinoda and Kushima who were kind enough to give me instruction and guidance.

References

1) Amano and Akiyama, Nippon Fujikagakkai Zasshi, 1925, **20**, 926.
2) Amano, ibid., 1928, **23**, 125.
3) Akiyama, ibid., 1927, **22**, 1190.
4) Eufinger, Zt. Geb. Gyn., 1929, **95**, 201.
5) Wada, Tohoku Igaku Zasshi, 1947, **36**, 83.
6) Hohlweg, Med. Klin., 1915, **11**, 331.
7) Shinoda, Nippon Fujikagakkai Zasshi, 1922, **17**, 809.
8) Nakai, ibid., 1926, **21**, 723.
9) Nakayama, ibid., 1932, **27**, 10, 11, 12.

10) Cobb-Bailey and Holtz, Amer. Jour. Physiol., 1917, **44**, 239.
11) Go, Tokyo Igakkai Zasshi, 1924, **38**, 703.
12) Iwata, Nippon Gekahokan, 1927, **4**, 813.
13) Tukahara, Nippon Seikeigekagakkai Zasshi, 1938, **8**, 156.
14) Takahashi, ibid., 1934, **9**, 222.
15) Miyoshi, Nippon Gekahokan, 1938, **15**, 53.
16) Kushima, Tohoku Igaku Zasshi, 1948, **37**, 27; Nippon Fujinkagakkai Zasshi, 1949, **44**, 107.